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MONITORING BALD EAGLES ON WISCONSIN'S GREAT LAKES

Grant recipient:

WISCONSIN DEPARTMENT OF NATURAL RESOURCES

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The Wisconsin Coastal Management Program, part of the Wisconsin Department of Administration, was established in 1978 to preserve, protect and and develop the resources of the Lake Michigan and Lake Superior coastline for this and future generations. The Wisconsin Coastal Management Program analyzes state policy on Great Lakes issues, coordinates government programs that affect the coast, and provides grants to stimulate better state and local coastal management.

WISCONSIN BALD EAGLE PRODUCTIVITY AND CONTAMINANT EXPOSURE
GREAT LAKES VS. INLAND NEST SITES
1990 - 1992

1992 FINAL REPORT

submitted to:

Wisconsin Coastal Management Program
Wisconsin Department of Administration
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EXECUTIVE SUMMARY

The International Joint Commission (IJC) Science Advisory Board has recommended using the bald eagle as a biosentinel of ecosystem health and water quality in the Great Lakes. The Wisconsin DNR is currently collaborating with Michigan State University (MSU), University of Wisconsin, University of Minnesota, and the National Park Service (NPS) to develop methodologies and a protocol for using the bald eagle as a biosentinel in the Great Lakes basin. The objective of this project is to enhance protocol development by measuring Wisconsin Great Lakes bald eagle exposure and relating exposure to recent reproductive history, and measures of fish and sediment contamination.

Plasma samples were collected from 97 Wisconsin bald eagle nestlings 1990-1992. Under this grant, 60 nestling blood plasma samples collected 1990-91 were analyzed for total PCBs (polychlorobiphenyls) and DDE (a breakdown metabolite of the insecticide DDT) at MSU Pesticide Research Center. All plasma samples (100%) contained detectable levels of total PCBs while 44 samples (73%) had detectable levels of p,p'-DDE [Instrument Detection Level (IDL) of total PCB = 5 ug/L, p,p'-DDE = 2.5 ug/L]. Plasma concentrations of total PCBs ranged from 18 - 330 ug/L while p,p'-DDE concentrations ranged from <IDL - 77 ug/L. The geometric mean nestling plasma total PCB concentration was 66 ug/L, the geometric mean p,p'-DDE plasma concentration was 6 ug/L.

The geometric mean plasma concentration of total PCBs was greatest in bald eagle nestlings sampled along the industrialized portions of the Wisconsin River (Rhinelander - Castle Rock Flowage) and lower Fox River (both rivers have historic PCB point sources; mean total PCB = 125 ug/L, n=11), followed by nestlings sampled along Lake Superior (mean total PCB = 84 ug/L, n=16), nestlings sampled at nests >8 km from the shoreline in counties adjacent to Lake Superior (mean total PCB = 47 ug/L, n=13) and in counties adjacent to Lake Michigan (mean total PCB = 46 ug/L, n=13), while the lowest mean concentration was found in nestlings sampled along lakes or rivers which have not received point source discharges and are remote from agricultural activity (total PCB = 33 ug/L, n=7). Between 77-100% of nestlings sampled along the Great Lakes shoreline and in adjacent counties had detectable levels of plasma p,p'-DDE. This compares to 58% of nestlings sampled on the Wisconsin River and lower Fox River, while only 17% sampled at inland sites remote from point sources had detectable levels of plasma p,p'-DDE. Nestlings sampled along the Lake Superior shoreline had the greatest geometric mean plasma concentration of p,p'-DDE (mean p,p'-DDE = 16 ug/L, n=16), followed by nestlings sampled at nests >8 km from the shoreline in counties adjacent to Lakes Superior (mean p,p'-DDE = 6 ug/L, n=13) and in counties adjacent to Lake Michigan (mean p,p'-DDE = 7 ug/L, n=13). Even lesser concentrations were found in nestlings sampled in inland counties (below point source discharge, mean p,p'-DDE = 5 ug/L, n=11; no point source, mean p,p'-DDE <2.5 ug/L, n=7).

There was no relationship between plasma total PCB and the reproductive history (# young hatched/nest attempt, 1985-1992) of the nest territory it was collected in (r^2 = 0.07, n=48), however the relationship was somewhat greater between plasma p,p'-DDE and nest territory reproductive history (r^2 = 0.22, n=48).

Bald eagles nesting in the region of Wisconsin which had the greatest mean plasma PCB concentration (the Wisconsin River/Fox River) have a healthy reproductive rate (1985-92 productivity = 1.36 $\pm$ 0.16 young hatched/active territory, 1992 = 21 territories) however many territories have existed <10 years and data indicates that reproductive performance of PCB-exposed eagles declines over time. Because loss of successfully breeding adults has a greater impact on bald eagle population dynamics than does nestling mortality, scientists at Michigan State University are developing an index of adult survival, a DNA fingerprinting technique which will allow for determination of adult turnover rates at nest sites. This data is required to accurately assess the impact of contaminants at a given nest territory.

Nestlings in the Lake Superior shoreline region had the greatest mean plasma p,p'-DDE concentrations and a lesser rate of productivity (0.94 $\pm$ 0.18 young/nest attempt, n=8 years) however prey availability appears to be limited in that region. Lake Superior nestlings received 72% fewer prey deliveries than did inland Wisconsin nestlings in 1992; the average prey delivery rate for the Lake Superior shoreline and Apostle Islands nests was 1.65 prey items/16 hours of observation while 5.90 prey deliveries/16 hours occurred inland. Of the identified prey items delivered to Lake Superior bald eagle nests, 80% were fish, 14% were birds, and 6% were mammals. This compares with inland prey deliveries which were 97% fish, 2% reptiles (turtles), 0.8% mammals, and 0.2% birds. The size class of the Lake Superior prey items include: 0-6" = 17%, 6-12" = 68%, 12-18" = 12%, and >18" = 3%. The size class of inland prey items include 0-6" = 4%, 6-12" = 61%, 12-18" = 33%, and >18" = 2%. Therefore, Lake Superior nestlings received not only 72% fewer prey deliveries but also smaller prey items (85% were <12" on Lake Superior, 65% were <12" inland). While fish dominated the diets of eaglets at both inland and Lake Superior nests, birds were a more common diet component on the Lake Superior shoreline (14%) than inland (0.2%).

Two nestling plasma samples collected from nests along the shoreline of Green Bay are currently undergoing chemical analysis at Michigan State University. This region has the poorest bald eagle reproductive history in Wisconsin and fish from the region have the greatest concentrations of PCBs and DDE in the state today.

PROJECT OBJECTIVES

Recently, the International Joint Commission Science Advisory Board has recommended using the bald eagle as an bioindicator of ecosystem health and water quality in the Great Lakes. The Wisconsin Department of Natural Resources (DNR) is among several regulatory agencies charged with establishing water quality criteria protective of wildlife in the Great Lakes. The Wisconsin DNR is currently collaborating with Michigan State University (MSU), University of Wisconsin, University of Minnesota, and the National Park Service to develop methodologies and a protocol for using the bald eagle as a biosentinel of Great Lakes ecosystem health. The primary objective of this project is to enhance protocol development by measuring Wisconsin Great Lakes bald eagle organochlorine (OC) contaminant exposure and relating exposure to recent reproductive history, and measures of fish and sediment contamination. Specific goals include increasing the number of Wisconsin nestling bald eagle plasma samples collected to 100 for 1990-1992 and to conduct chemical analysis of 60 plasma samples. The samples were analyzed for total PCBs (polychlorobiphenyls) and p,p'-DDE (a breakdown metabolite of the insecticide DDT, dichlorodiphenyltrichloroethane) at MSU Pesticide Research Center. These contaminants have been implicated in reducing bald eagle reproductive performance in the Great Lakes basin and are known to cause eggshell thinning, embryo mortality, and teratogenicity. Results of this analysis will be used to provide baseline data regarding Wisconsin Great Lakes bald eagle nestling OC exposure that exposure will be compared to that of nestlings further inland. In addition, the Wisconsin bald eagle nestling plasma total PCB and p,p'-DDE concentrations were related to the reproductive history (1985-92) of the nest territories at which the samples were taken. Finally, eaglet plasma contaminant concentrations were compared to the contaminant levels of game fish and sediment samples taken in the region of the nest sites. Lake Superior bald eagle foraging behavior and nesting ecology were also documented and 1992 Wisconsin Great Lakes bald eagle productivity was measured.

BACKGROUND

Industrial and agricultural pollution has contaminated the sediment and water column of several Wisconsin Great Lakes harbors and tributaries. Despite the ban on industrial uses of PCBs and agricultural use of the pesticide DDT in the 1970s, these substances persist in the Great Lakes foodweb and pose a health risk to humans consuming some Great Lakes fish. In response, the Wisconsin DNR has issued fish consumption advisories for sport fisherman along portions of Wisconsin's Great Lakes shoreline. Similar advisories have been issued for portions of the Menominee, Fox, Sheboygan, and St. Louis Rivers which discharge into the Great Lakes. While these compounds pose a health risk to humans, it is also suspected that they negatively impact the health and productivity of top-predator wildlife which are dependent on the Great Lakes foodweb.

Bald eagles (*Halieeatus leucocephalus*) nesting in the Great Lakes coastal region (<8 km from the shoreline) are less productive and experience greater contaminant exposure than do bald eagles which nest further inland (International Joint Commission, 1989). Consumption of contaminated prey from the Great Lakes (primarily fish) is the likely route of contaminant exposure. Kozie and Anderson (1991) found that Wisconsin Lake Superior bald eagle productivity was reduced, and nestling mortality and contaminant exposure was greater when compared to Wisconsin bald eagles nesting inland. They concluded that consumption of contaminated prey (primarily herring gulls) was the likely cause of reduced Lake Superior productivity. A more recent analysis of Wisconsin bald eagle productivity (1985-1992) showed that 20 active bald eagle nests were located within 8 km of the Wisconsin Great Lakes shoreline (8 km is thought to be the feeding range of nesting bald eagles). During that time, eagles nesting on Wisconsin's Lake Superior shoreline produced 0.94 young/nest attempt (n=110 nest attempts) while eagles nesting on the Lake Michigan shoreline produced 0.27 young/nest attempt (n=11 nest attempts). In contrast, eagles nesting in the North Central District of Wisconsin (all nests are >40 km from the Great Lakes) produced 1.32 young/nest attempt (n=1011 nest attempts). Productivity of 0.7 young/active nest is required for population stability (Sprunt et al. 1973) and 1.0 young/active nest is considered typical productivity for a "healthy" eagle population.

Unhatched bald eagle eggs collected from Wisconsin Great Lakes eagle nests 1970-1988 had total PCB, p,p'-DDE, and dieldrin concentrations 6 - 50x greater than eggs collected inland (Wiemeyer et al. 1984; WDNR unpubl. data). Blood plasma collected from 5 Wisconsin bald eagle nestlings in 1989 contained PCB and DDE levels 5x greater than plasma collected from nestling eagles at inland sites (Bowerman et al. 1989). Bald eagle nestling blood plasma provides a "top of the food chain" measure of contaminant exposure specific to a given site because nestling plasma contaminant levels likely reflect that of prey collected within the adult feeding territory (generally within 8 km of the nest).

METHODOLOGY

Blood Plasma Collection

A total of 97 nestling plasma samples were collected at Wisconsin bald eagle nest sites 1990-92 (Figures 1,2,& 3). Contracted climbers or WDNR personnel (D. Evans, Hawk Ridge

Observatory, Duluth, MN; R. Eckstein, WDNR) captured bald eagle nestlings by climbing to the nest and restraining the nestlings when they were 4-7 weeks old. The climber placed a US Fish and Wildlife Service band on the nestling and lowered it to a ground crew in a nylon bag. On the ground, two field technicians weighed the eaglet and drew ten cc's of whole blood from the bracialus vein. One technician held the eaglet and secured the talons while the other drew blood. Ten cc's of blood were obtained with sterile 10 cc syringes (glass or plastic) tipped with 20 ga needles. The whole blood was placed in a 10 cc heparinated vacutainer for organochlorine pesticide and total PCB analysis. All blood containers were then labelled with the bird's band number, nest location, state, date, and initials of the technicians present. Three contour breast feathers were collected from the nestling for mercury analysis. Six measurements were obtained from each nestling to determine their age and sex; hallux claw arc, foot pad length, eighth primary feather length, sixth tail feather length, culmen depth, and length of the gape. The nestling was then returned to the nest.

Blood samples were placed on ice and refrigerated upon return from the field. Within 48 hours the whole blood was centrifuged for 10 minutes at 1200 rpm and plasma was transferred to another green top vacutainer with a clean pipette. This vacutainer was labeled as above and frozen upright. The red blood cells were removed from the vacutainer and placed in a centrifuge tube. The empty green top vacutainer was rinsed with normal saline solution into the centrifuge tube. The centrifuge tube was then spun for 10 minutes. The saline layer was decanted off using a clean pipette and 5 ml of saline solution was added to the tube. The tube was centrifuged for an additional 5 minutes and the saline layer decanted off again. At this point 0.1 ml of buffer was added and the centrifuge tube was labeled and frozen upright. At the end of the field season all frozen samples were shipped on dry ice via Federal Express to Michigan State University for contaminant analysis.

Samples were collected in different regions to compare bald eagle nestling exposure in various Wisconsin habitats. First, nestling plasma was obtained from all productive eagle nests along Wisconsin's Lake Superior and Lake Michigan shorelines. Second, nestling plasma was collected from a sample of eagle nests in counties adjacent to the Great Lakes (Douglas, Bayfield, Ashland, Iron, Marinette, Oconto Co.) but > 8 km inland (these eagles are unlikely to feed directly on the Great Lakes). Finally, nestling plasma was collected in inland counties (Vilas, Oneida, Lincoln, Marathon, Portage, Wood, Adams, Juneau, Sawyer, Rusk, Shawano, Outagamie) at two types of sites. One type of habitat included riverine nests along the Wisconsin and Fox Rivers that are near point source discharges from industry and municipalities. The second type of inland site is nests which occur on waterways which receive no point source discharge within 40 km of the nests.

Blood Plasma Analysis

Concentrations of organochlorines were determined by gas chromatography with confirmation of pooled samples by mass spectrometry at MSU Pesticide Research Center (Price et al. 1986; MDPHL 1987). Individual 2-4 ml plasma samples were dissolved in methanol and extracted twice with 5 ml of a 1:1 mixture of hexane-ethyl ether by agitating on a rotary mixer for 20 minutes at 50-55 rpm. Extracts were concentrated on a hot water bath to a volume of 0.5 ml. Clean-up was done on a 7 mm Chromaflex column packed with 2.5 g of Florisil using 10 ml of hexane. Elution of polycholorinated biphenyls (PCBs) and chlorinated hydrocarbon pesticides from the Chromaflex column was accomplished with 20 ml of 6% ethyl ether/hexane. Elution of dieldrin from the column was accomplished with 20 ml of 20% ethyl ether/hexane. Separation of PCB from the chlorinated hydrocarbon pesticides was accomplished with a Chromaflex column packed with Silica Gel 60. Elution of hexachlorobenzene (HCB) and mirex was accomplished with 15 ml hexane. Elution of Aroclor 1260, Aroclor 1016, and polybrominated biphenyl (PBB) was accomplished with an additional extraction with 20 ml hexane. Elution of Aroclor 1016 and chlorinated hydrocarbon pesticides was accomplished with 20 ml of benzene (MDPHL 1987). Gas chromatography was performed on a Varian 3700 gas chromatograph equipped with small volume pulsed ^{63}Ni electron capture detectors, Varian 8000 Auto Sampler, and CDS-111 microprocessor. A 1.83 m x 0.64 cm x 2 mm i.d. glass column packed with 3% SE-30 was used. Nitrogen flow rate was 30 ml/min through the column during operation. Total PCB concentration was determined on the basis of mean weight percent factors (Webb and McCall 1983) and other individual peaks were determined by reference to the relative retention time of p,p'-DDE x 100 (MDPHL 1987). We measured concentrations of total PCBs (measured as Aroclor 1254 or 1260), 1,1'-(2,2,2-Trichloroethylidene)bis[4-chlorobenzene] (p,p'-DDT), and its metabolites p,p'-DDD and p,p'-DDE, HCB, heptachlor epoxide, cis-nonachlor, trans-nonachlor, oxychlordane, dieldrin, PBB, toxaphene, mirex, alpha-chlordane, and gamma-chlordane. (See Appendix for Standard Operating Procedure and certification of results by MSU QA/QC officer).

Food Habits and Foraging Behavior

Food habits of nesting bald eagles have often been determined by collecting prey remains in the nest and on the ground around the nest tree and quantifying the prey items present. In a recent study (Mersmann et al. 1992) it was concluded that this technique is biased towards over-representation of birds, medium sized mammals, and large bony fish while small mammals and small fish were under-represented. Because of the bias, two additional techniques were used to determine Wisconsin Great Lakes bald eagle food habits.

Technicians conducted dawn-to-dusk observations at several Lake Superior shoreline nests to document bald eagle food habits while video cameras mounted at nest sites were used to document eagle feeding behavior at inland nest sites.

Direct Observation Protocol

Observations of bald eagle nesting and feeding behavior were conducted at seven nests on the Wisconsin Lake Superior shoreline, including two on the Apostle Islands National Lakeshore (APIS), during the 1992 nesting season. Observations were conducted at all known nest sites within 8km of the shore of Lake Superior where suitable blind sites were available. Location of the nests is shown in Figure 3 and include DU-9b (T45N R10W S18), BY-23 (T49N R09W S05), BY-21a (T51N R7W S35), BY-3b (T52N R4W S16), BY-15b (T47N R5W S08), AS-20c (T51N R1W S14), and IR-32 (T47N R1E S12).

Direct observations were made with binoculars and spotting scopes. Scopes included a Nikon Field Scope, a Kowa TSN-1, a Bushnell Spacemaster, a Celestron SS-80, and a Meade 97E. All scopes had magnifications ranging from 20x-60x with 40x the most common selection for viewing the nests from the distances of the blinds. Incubation observations were conducted at four nests in April. After hatching, 24 hour observation periods began; 1300 - dusk on day 1, dusk - dawn on day 2. Each observation shift was approximately four hours long for a single observer, observers then switched. Training of observers was conducted by an experienced observer familiar with the procedure. Every five minutes the observer would enter a code descriptive of the major activity of each eagle on the territory during the previous five minutes. Food habits were closely monitored; time of prey delivery, prey type, and size class were recorded.

Video Camera Protocol

Six video cameras were mounted February 1-15, 1992, at bald eagle nest sites in northern Wisconsin (Figure 3) before the initiation of nesting. Two cameras were mounted in nest trees and four were mounted in trees adjacent to the nest tree. Three territories were on the Turtle Flambeau Flowage, Iron County (IR-9c, IR-33, IR-35a), one was on a lake in Vilas County (VI-61c), and two were on lakes in Oneida Co (ON-49, ON-79). All six territories produced chicks in 1992. Cameras were camouflaged with natural materials to minimize their visual impact. Spruce and balsam fir bows were attached to 1" chicken wire and wrapped around the Sony M-350 black and white video cameras, pine boughs and cedar bark were used to camoulflage the smaller M-332 Sony cameras. Coaxial cable (type RG-8UM, Tandy Corp.) connected the video cameras to Sony 8mm time lapse recorders modified for field use by Fuhrman Diversified, Laporte, TX. Recorders were placed 200-300m from the nest tree to minimize disturbance to the nest site when batteries and tape were changed. The cable was anchored to the trunk of the tree with 1" electric staples as cable run on the ground was damaged by white-tailed deer and snowshoe hare in the spring. An 8mm pseudo-time lapse video recorder (model WCMS-4/V11, Fuhrman Diversified, Inc.) was loaned to the research project from the National Park Service and 2 Fieldcam TLV 8mm timelapse video recorders (Fuhrman Diversified, Laporte, TX) were purchased by the WDNR with grant support from the Great Lakes Protection Fund. The recorders were set to record 1 frame/second and tape and batteries were changed every 3-4 days. Fuhrman Diversified manufactured customized weatherproof housing for the Sony recorders and modified the recorders so that they could be powered with 12 V DC batteries. When operated in the field the recorders and batteries were placed in custom security boxes constructed of 16 gauge steel sheet metal which were hinged, padlocked, and chained to large trees. An internal time/date stamp superimposed the date and time over the video image. Video recordings were then analyzed using a video monitor and bald eagle time/activity budgets, food habits (time and number of daily prey deliveries, prey size, prey type), and behavior were quantified and compared to data gathered along the Lake Superior shoreline.

Nest Prey Remains

Prey remains were collected at all nest sites that nestling plasma was obtained. Bones, scales, and feathers will be identified to provide an estimate of the type of prey delivered to the chicks at the nest site. This information will provide an index with which to assess the primary routes of contaminant exposure at a particular nest site. Analysis of material is underway at Michigan State University.

Great Lakes vs. Inland Productivity

Two aerial surveys, one conducted in mid-April and one in late-May, were used to obtain detailed information on the Wisconsin Lake Superior and Lake Michigan bald eagle population status (number of occupied territories) and reproductive performance (% nest success, number of young produced/occupied bald eagle territory). An additional flight was conducted on May 8 to identify nests which had been abandoned without producing young. A climber entered these nests and searched for unhatched eggs for contaminant analysis. In addition, all nests were revisited when chicks were 4-6 weeks of age to collect nestling plasma samples. In order to continue yearly comparisons of inland and Wisconsin Great Lakes bald eagle productivity, the WDNR conducted two aerial surveys of all bald eagle territories in Wisconsin's North Central District, approximately 150 territories representing 30-40% of the state's total breeding population.

Fish and Sediment Contaminant Data

All available data was gathered on the concentrations of contaminants in fish and sediment in the general regions that bald eagle nestling plasma was collected. Primary data sources included the Wisconsin DNR Fish Contaminant Database, the Green Bay Mass Balance Project, and the WDNR Bureau of Water Resource Management.

RESULTS & DISCUSSION

1990 - 1991 Wisconsin Nestling Plasma Contaminant Concentrations

Sixty Wisconsin bald eagle nestling plasma samples collected 1990-91 were analyzed for total PCB and p,p'-DDE content at Michigan State University Pesticide Research Center. All plasma samples (100%) contained detectable levels of total PCBs while 44 samples (73%) had detectable levels of p,p'-DDE [Table 1; Instrument Detection Level (IDL) of total PCB = 5 ug/L, p,p'-DDE = 2.5 ug/L]. Plasma concentrations of total PCBs ranged from 18 - 330 ug/L while p,p'-DDE concentrations ranged from <2.5 (IDL) - 77 ug/L. The geometric mean nestling plasma total PCB concentration was 66 ug/L, while the mean p,p'-DDE plasma concentration was 6 ug/L.

Samples were categorized according to region of collection (as described in methods section) and the frequency of detection and geometric mean plasma contaminant concentrations were compared. As stated previously, PCBs were detected in the plasma of 100% of nestlings sampled in all regions of Wisconsin. The greatest geometric mean plasma concentration of PCBs was found in bald eagle nestlings sampled along rivers with historic point sources of PCBs [the industrialized portion of the Wisconsin River (Rhinelander - Castle Rock Flowage)and the lower Fox River](mean total PCB = 125 ug/L, n=11, Table 2, Figure 4), followed by nestlings sampled along Lake Superior (mean total PCB = 84 ug/L, n=16), nestlings sampled at nests >8 km from the shoreline in counties adjacent to Lake Superior (mean total PCB = 47 ug/L, n=13) and in counties adjacent to Lake Michigan (mean total PCB = 46 ug/L, n=13), and the lowest mean concentration was found in nestlings sampled along lakes or rivers which have not received point source discharge and are remote from agricultural activity (total PCB = 33 ug/L, n=7). Between 77-100% of nestlings sampled along the Great Lakes shoreline and in adjacent counties but >8 km inland had detectable levels of p,p'-DDE in their plasma. This compares to 58% of nestlings sampled at inland sites subject to historic point source discharge while only 17% sampled at inland sites remote from point source had detectable levels of p,p'-DDE (Table 2, Figure 5). Nestlings sampled along the Lake Superior shoreline had the greatest geometric mean concentration of p,p'-DDE in their plasma (mean p,p'-DDE = 16 ug/L, n=16), followed by nestlings sampled at nests

>8 km from the shoreline in counties adjacent to Lakes Superior (mean p,p'-DDE = 6 ug/L, n=13) and in counties adjacent to Lake Michigan (mean p,p'-DDE = 7 ug/L, n=13). Even lesser concentrations were found in nestlings sampled in inland counties (below point source discharge, mean p,p'-DDE = 5 ug/L, n=11; no point source, mean p,p'-DDE <IDL), n=7).

While nestlings sampled along Lake Superior had mean total PCB concentrations nearly 2.6x greater than nestlings sampled at remote inland Wisconsin sites, the mean concentration was less than half the mean found for other Great Lakes bald eagle nestling plasma samples (Bowerman et al. 1990, Table 3), and less than concentrations of PCBs in plasma collected along the industrialized rivers of Wisconsin, and along the lower Columbia River in Oregon/Washington (Table 4). The relatively small concentration of PCBs in Lake Superior nestling plasma is compatible with fish contaminant data, only large lake trout have detectable levels of PCBs along Wisconsin's Lake Superior shoreline. Piscivorous avian species, such as herring gulls, do have elevated levels of PCBs in Lake Superior, are taken by eagles, and may account for PCB concentrations of 100-201 ug/L found in 7 of 16 (44%) Wisconsin Lake Superior eaglet plasma samples; 4 of the 7 samples were collected on the Apostle Islands archipelago. Eagles were observed delivering birds to nestlings at all Lake Superior nests we observed in 1992. In comparison, total PCB concentrations exceeded 100 ug/L twice as often (91%) in samples collected on the industrialized portions of the Wisconsin and lower Fox River where many fish, particularly carp, have detectable concentrations of PCBs. Some Lake Superior eaglets appear to consume little contaminated prey as 25% had plasma concentrations within the range (24-66 ug/L) found in nestlings in remote Wisconsin nest locations. In addition, there is evidence of great year to year variability in exposure on Lake Superior, indicating that contaminant exposure may be sporadic. While the 1990 nestling plasma PCB concentration at AS-12 (41 ug/L, N. Twin Island, APIS) was among the lowest in the state, a nestling sampled in the same nest in 1991 had total PCB levels 5x greater (201 ug/L). It is possible that it had fed solely upon fish in 1990 but had fed upon gulls (or other contaminated birds) in 1991. The mean total PCB content of nestling plasma samples collected along the Wisconsin River and lower Fox River was nearly identical to that found along the lower Columbia River in Oregon/Washington; these rivers all receive effluent from pulp and paper mills which often contained PCBs in the 1960s and 1970s (primarily due to de-inking processes). The greatest fish and sediment PCB levels found today in Wisconsin occur in portions of the lower Fox River and in lower Green Bay. Two nestlings were sampled in a nest on the lower Fox River (in Kaukauna) and were found to have elevated plasma PCB levels (115 & 124 ug/L). Even greater concentrations were found in eaglets at inland sites in Marinette Co. (213 ug/L lower Menominee River location, 330 ug/L Lake Noquebay) and from eagles on the Wisconsin River (187, 197, & 277 ug/L from eaglets on the Petenwell/Castle Rock Flowages;

146 & 157 ug/L in nestlings from Lincoln and Marathon Co.). The nests in Marinette County were both within 12 miles of the Green Bay shoreline and it is conceivable that the nestlings were fed fish, or avian species which feed on fish, from Green Bay. The eagle nest on the lower Fox River lies midway between the two major sediment sources of PCBs on the river and it is possible that the locks and dams compartmentalize the sediment thus protecting the eaglets from receiving the greatest dose of PCBs possible on that river system. A plasma sample was collected from a nestling in a nest on the mouth of the Oconto River near the shore of Green Bay (Lake Michigan) in 1990. The sample was placed in the wrong batch at the analytical laboratory and will be analyzed in January 1993 as will an additional Green Bay shoreline plasma sample collected in 1992 in Marinette Co. It is likely that both samples will have greatly elevated total PCB concentrations as most Green Bay fish still contain >1 ppm total PCB.

Nearly all nestlings (86%) sampled in counties adjacent to the Great Lakes had detectable p,p′-DDE in their plasma, however the metabolite was detected in only 44% of samples collected in inland Wisconsin counties. The greatest concentrations of p,p′-DDE were found in Lake Superior nestling plasma samples; only 9 Wisconsin samples exceeded 20 ug/L, however 7 of 9 samples (78%) were collected along Lake Superior. This finding is consistent with the likelihood that most OC pesticide residues have been metabolized and/or transported from the inland rivers, however a slower rate of metabolism and longer residence time is suspected for DDT and DDE in the Great Lakes, particularly Lake Superior. In addition, some regions near Wisconsin′s Great Lakes shoreline were placed into fruit orchards (particularly Door and Bayfield Co.) and received heavy OC pesticide application before the compounds were banned in the 1970s, some of which persists in the offshore and tributary sediment. The mean p,p′-DDE concentrations for all regions in Wisconsin, including Lake Superior, were much less than that found for other Great Lakes nestlings (Table 3) and those on the lower Columbia river (Table 4). Despite the fact that they had comparable levels of PCBs, the mean p,p-DDE concentration of plasma samples collected on the Wisconsin/Fox River (4 ug/L) was 96% less than that found in lower Columbia River plasma samples (98 ug/L). Samples collected from remote Wisconsin sites also contained lesser concentrations than those collected in the Michigan and Oregon interior.

There was no relationship between plasma total PCB and the reproductive history (#young hatched/nest attempt, 1985-1992) of the nest territory it was collected in (r^2 = 0.07, n=48; Figure 6), however the relationship was somewhat greater between plasma p,p′-DDE and nest territory reproductive history (r^2 = 0.22, n=48; Figure 7). Bald eagles nesting in the region of Wisconsin with the greatest mean plasma PCB concentration (the Wisconsin River/Fox River) have had excellent productivity (1985-92 productivity = 1.36 $\pm$ 0.16 young hatched/active territory, 1992 = 21 active territories) however many have been occupied <10 years and data indicates that reproductive performance of PCB-exposed eagles declines over time. Because loss of successfully breeding adults has a greater impact on bald eagle population dynamics than does nestling mortality, Michigan State University is developing an index of adult survival, a DNA fingerprinting technique which will allow for determination of adult turnover rates at nest sites. This data is required to accurately assess the impact of contaminants on a given nest site. The region where nestlings had the greatest mean plasma p,p'-DDE concentrations (Lake Superior nestlings) had poorer productivity (1985-92 productivity = 0.94 $\pm$ 0.18 young/nest attempt, 1991 = 18 active territories) however prey availability also is limited in that region (see Foraging and Food Habits. Two plasma samples collected from Green Bay are currently undergoing analysis; this region has the poorest bald eagle reproductive history in Wisconsin and fish from the region have the greatest concentrations of PCBs and DDE in the state today.

1992 Nestling Plasma Collection

In 1992 we collected blood and feather samples from 33 bald eagle nestlings, including all 11 productive nests within 8 km of the Great Lakes (Table 5). The full 10 ml of blood was collected from all but one nestling from which 9 ml were collected. Three contour breast feathers were collected from 30 nestlings, two feathers from two nestlings and one nestling had not yet developed contour feathers so none could be collected. Sample analysis is currently being conducted at the laboratory of Dr. John Giesy, Michigan State University Pesticide Research Center with grant support from the Great Lakes Protection Fund.

Foraging and Food Habits

Direct Observations

Direct observations were conducted at seven bald eagle nests on the Wisconsin Lake Superior shoreline and the Apostle Island National Lakeshore (APIS) in 1992. Direct observations were initiated 8 April with an incubation observation at IR-32a. A total of 72 hours of incubation observations and 872 hours of post-hatching direct observations were made on the Wisconsin Lake Superior shoreline. Observers witnessed 92 prey deliveries. Eggs failed to hatch at one nest while two nests failed after hatching (see 1992 Great Lakes Productivity below).

Video Recordings

Eighty one hours of incubation activity was recorded at three territories from late March - mid April. Nearly 2800 hours of nest activity was recorded during the post-hatching stage. A total of 601, 627, and 766 hours of nest activity was recorded at the IR-9a, IR-33, and ON-79 territories, respectively. These sites were monitored continuously through the nesting season. Recording began at ON-49 on 2 June because a camera adjustment was required (the eagles had added 50 cm of nest material to the nest, placing the camera out of position). A total of 308 hours of nest behavior was recorded at ON-49. Final camera adjustments were also made at the IR-9a and IR-33 nests the last week in May as portions of those nests were also not visible. The camera at VI-61c was moved to the new nest 2 June and recording began at that time; 467 total hours were collected at VI-61c. A camera was also mounted at IR-35c, 15 June. This camera was only operated for two weeks as only 70% of the nest was visible and 110 hours of nest activity was recorded.

Quantity of Prey Delivered

The prey delivery rate was calculated for each Lake Superior nest by totalling the number of prey deliveries, dividing by the total number of hours observed at that nest and multiplying by 16 to normalize the rate to a 16 hour daylight period. Prey delivery rates were 1.62, 1.40, 2.06, 1.75, and 1.36 prey items per 16 hours of observation at nests BY-23 (2 yng), AS-20c (2 yng), BY-15a (2 yng, 1 died), IR-32a (2 yng) and BY-21 (2 yng, both died), respectively. The average prey delivery rate for the Lake Superior shoreline and Apostle Islands nests was 1.65 prey items per 16 hours of observation (Figure 9).

The prey delivery rate was also calculated for inland nest sites by totalling the number of prey deliveries recorded on video tape, dividing by the total number of daylight hours video recordings occurred, and multiplying by 16 to normalize the rate to a 16 hour daylight period. Prey delivery rates were 6.87, 5.43, 7.03, 7.53, and 1.86 prey items per 16 hours of video recordings at nests ON-79 (2 yng), ON-49 (1 yng), IR-33 (3 yng), VI-61c (3 yng), and IR-9a (2 yng, 1 died) respectively. One nestling died at IR-9a (lowest inland prey delivery rate) while all nestlings survived at the other nest sites. The average prey delivery rate for the inland nest sites was 5.91 prey deliveries/16 hours of video recording (Figure 9).

Type and Size of Prey Delivered

Ninety two prey deliveries were documented at five successful Lake Superior bald eagle nests in 1992. Observers were able to identify 64 of the prey items delivered (70%) to class (fish, mammal, bird, reptile; Figure 10) and were able to determine the size class (0-6", 6-12", 12-18", >18"; Figure 11) of 66 prey items (74%). The video cameras mounted at inland Wisconsin bald eagle nests recorded 694 prey deliveries at five successful nests in 1992. Of the known prey items delivered to Lake Superior bald eagle nests, 80% were fish, 14% were birds, and 6% were mammals. This compares with inland prey deliveries which were 97% fish, 2% reptiles (turtles), 0.8% mammals, and 0.2% birds. The size of the Lake Superior prey items include 17% in the 0-6" size class, 68% were 6-12", 12% were 12-18", and 3% were >18". The size class of inland prey items include 4% between 0-6", 61% were 6-12", 33% were 12-18", and 2% were >18". Therefore, Lake Superior nestlings received not only 73% fewer prey deliveries but also smaller prey items (85% were <12" on Lake Superior, 65% were <12" inland). While fish dominated the diets of eaglets at both inland and Lake Superior nests, birds were a more common diet component on the Lake Superior shoreline (14%) than inland (0.2%).

Nest Prey Remains

Nest prey remains were collected by the climber while the nestlings were being bled. The ground crew also collected all prey remains visible at the base of the nest tree. Samples are currently being analyzed at Michigan State University.

1992 Wisconsin Great Lakes Bald Eagle Productivity

Nest success on the Wisconsin Lake Superior shoreline (nests within 8 km of the shoreline) in 1992 was 67%; 8 of 12 nest attempts produced young. Thirteen bald eagles were fledged from the 12 active territories for a productivity of 1.08 young/active territory (Figure 12) and 1.62 young/successful nest. This compares to 11 successful nests out of 18 active territories (61%) in 1991, with 17 young produced (0.94 young/active nest, 1.54 young/successful nest). In contrast, both previously active nests on Wisconsin's Lake Michigan (Green Bay) shoreline did not produce young in 1992. A climber entered both nests and found the one near the mouth of the Oconto River (OC-4) had been worked on but there were no indications that eggs were laid (D. Evans, pers. comm.). A raptor biologist who lives nearby reported that both adults were seen at the nest in early spring (T. Erdman, pers. comm.). The other territory is near the mouth of the Peshtigo River (MT-7 and 7a) and was not worked on in 1992 (D. Evans, pers. comm.) however one adult was observed on the territory in the spring (T. Erdman, pers. comm.). Two new nests were located on Green Bay in 1992. One nest in Marinette County produced 1 young (plasma was sampled) and one unhatched egg was collected. The other nest was constructed on Little Tail Point in Brown County, no eggs were laid, however 1 adult and 1 subadult were in residence (T. Erdman, pers. comm.)

Inland Wisconsin productivity (Northcentral District) in 1992 remained good, 139 out of 176 active territories produced a total of 233 young; nest success = 79%, productivity = 1.32 young/active territory (Figure 12), 1.68 young/successful territory. In 1991, inland Wisconsin productivity (Northcentral District) included 72% nest success (111 out 155 active territories produced 190 young) and productivity rates of 1.23 young/active territory, and 1.71 young/successful nests.

Statewide, the number of active bald eagle territories increased from 418 in 1991 to 424 in 1992.

Nest Abandonment, Nest Failures, and Nestling Mortality - 1992

Although nest success on the Lake Superior shoreline was good in 1992, there were several territories, particularly on the APIS and in Chequamegon Bay, which were initially occupied but abandoned prior to egg laying. This abandonment of nesting effort is interesting due to the relatively close proximity of all nests involved. Several territories on the APIS (Basswood Is., Oak Is., Outer Is., Long Is., and Sand Point) had adults present and nest construction was documented. At four of the five territories a climber entered the nest, after the adults had abandoned, and observed fresh grass and the beginning of a nest cup but found no eggs (D. Evans, pers. comm.). In addition, nests at Madeline Is., Oak Point (AS-18)and Honest John Lake (AS-21) also had adults on territory but were not observed incubating during the spring flights. We were unable to enter these nests to ascertain their condition because they lie within the Bad River Reservation (Ojibway) who have not given the state permission to climb nests on their territory. All nests mentioned, with the exception of Outer Is., are in close proximity of Chequamegon Bay and the eagles would likely use the bay as a primary feeding area. In the spring of 1992 ice cover was completely off of Chequamegon Bay on 23 April, later than normal (Tom Doolittle, NPS, pers. comm.). The eagles on the Chequamegon Bay also rely on commercial fisherman to provide food during the early spring months when nest initiation takes place; rough fish are thrown on the ice as nets are retrieved and eagles frequently take these fish. In the winter of 1991-1992 there was a 56% reduction in commercial fishing effort through the ice on Chequamegon Bay (Tom Doolittle, pers. comm.), potentially reducing the available food to the eagles returning to the area. The water surrounding the peripheral islands (i.e. Devils Is., North Twin Is., Outer Is., and Michigan Is.), on the other hand, did not freeze over last winter, allowing eagles on territory constant access to their prey base (spawning burbot and lake herring) during the early nesting period (March-April). The reduction of a readily available food supply may have contributed to the reduced nesting effort on territories in close proximity to Chequamegon Bay in 1992.

Three nests on the Lake Superior shoreline where direct observations had taken place failed at some point in the nesting season. The nest on the mouth of the Brule River (DU-9a) was observed empty on 9 May when the hatchlings were three days old. A climber entered the nest after it had been noticed empty and could find no evidence of young, nor evidence of any predator having been in the nest. The second confirmed failure was at BY-3b (York Island) where the adults were observed incubating as late as 27 May, > 40 days after they were first observed incubating. Analysis of the observation data indicated no obvious lack of nest attentiveness or disturbance as incubation took place 98.3% of the time observed. Periodic checks were made of the territory and by mid-June no adults were seen. A climber entered the nest on 15 June and collected one addled egg for contaminant analysis. The final nest failure on the Lake Superior shoreline occurred in two stages. At nest BY-21 (Bark Bay) observations began on 18 May with two young in the nest. On 7 June a climber entered the nest to collect nestling serum samples and noted that only one chick was present. A few eaglet feathers were on the ground and in a crotch in the tree about 3 m below the nest. The remaining eaglet was banded and a blood sample was taken. During the observation period the following week, 8 June, observers noted no chick activity in the nest and reported no prey deliveries. On 11 June the climber reentered the nest, found the eaglet was missing. After a short search the remains of the banded nestling were found on the ground within 30 m of the nest tree. Remains included the head and beak, one foot (including the band), and numerous feathers, the rest of the carcass had been removed from the scene. The climber (D. Evans, raptor biologist with >20 years experience studying and banding bald eagles) concluded that the death was likely the result of mammalian predation. It was noted that the nest bowl had been dug into, consistent with raccoon predation. The fact that the banding team had visited the nest on 7 June and within 24 hours it had been predated seems to suggest an effect of some sort by the banding of the nestling. It has been documented by video camera and direct observation at 5 nests this year that adults do not return to the nest until the day following nest entry by a climber. It is possible that the banding crew provided a scent trail to the nest tree and lack of adult attentiveness allowed predation to occur. It should be noted that one nestling had disappeared 10 days prior to the loss of the second chick. It should be emphasized that there is no evidence that nest entry poses a general hazard to nesting bald eagles; in Wisconsin bald eagles have increased exponentially despite the fact that every nest in the state was entered and chicks banded from 1974-1989. However, individual nests may be placed at greater risk if the local predator population is high. The fact that adults do not return to a nest until the day after a climber has been there also means that harsh weather can claim chicks if they are banded before they are able to thermoregulate (week 3-4) or during extremely inclement weather. A fourth nest failure occurred at North Twin Island (APIS), no observations were made at this nest site and the cause of failure is unknown.

One nest, BY-15A (Fish Creek), contained two young at the beginning of observations, but only one remained on 28 May at the beginning of an observation period. The observations continued on a weekly basis with one chick in the nest when, on 25 June at 0739, an observer noticed a fisher (Martes pennanti) climbing the nest tree. The adults returned to the territory scolding and circling around the nest. The fisher retreated down the tree. The remaining eaglet at this nest fledged on 9 July. It is possible that the fisher was responsible for the loss of the nestling noticed missing on 28 May. Observers have speculated that the expanding fisher population may be impacting raptors through nest predation in northern Wisconsin.

A minimum of 5 of 18 nestlings known to have hatched on Lake Superior died before fledging (28%). Only 1 nestling out of 11 hatched was lost during the nesting season at the inland camera study sites (9%). That mortality occurred at IR-9a on the Turtle Flambeau Flowage. It died between week 1-2 of age and was observed being out-competed for food by its more aggressive nest mate which was nearly twice its size. This nest had a much lower prey delivery rate (comparable to the rate at Lake Superior nest sites) than did the other inland nests, the adults were present at the nest site much less frequently, and nestling mortality occurred at the nest on at least two other occasions in the past 7 years.

Nestling plasma, fish, and sediment contaminant levels

Bald eagle nestling plasma, fish, and sediment PCB data are presented in Table 6. The sediment and fish PCB data presented is that available for feeding areas within 10 miles of sampled eagle nests on portions of the Wisconsin River, the Menominee River, the lower Fox River, Duluth/Superior Harbor, Lake Superior shoreline (including the Apostle Islands), and the Flambeau River (WDNR Water Resource Management, unpubl. data). Most sites sampled had known PCB contamination therefore there is not a wide gradient in fish/sediment/plasma PCB concentrations.

The lower Fox River (Kaukauna) had the greatest concentrations of PCB in sediment and fish yet the nestlings had only intermediate plasma PCB concentrations (Table 6). Fish from the Flambeau River did not have detectable levels of PCBs (<0.2 ppm) and nestling plasma PCB levels were low in samples collected nearby, however no detectable PCBs were found in sediment or fish within 10 miles of an eagle nest on the Wisconsin River yet the nestling plasma PCB concentrations was 147 ug/L. Fish collected near the outer islands of the Apostle Islands (primarily lake trout) had greater concentrations of PCBs than did fish from any other region along Wisconsin's Lake Superior shoreline, however Duluth/Superior harbor came in a close second (Table 6). The greatest concentrations of nestling plasma PCBs found along Wisconsin's Lake Superior shoreline occurred in some nestlings from the region (1991 North Twin and Outer Island) however other nestlings had low plasma PCB concentrations. Of the fish sampled, only large lake trout had elevated PCBs, forage fish such as lake herring, lake whitefish, and burbot did not. A potential route of PCB exposure for these nestlings is that they are being fed commercial fishing waste (i.e. lake trout offal dumped overboard) or herring gulls and/or other colonial nesting birds or diving ducks known to have elevated PCB levels.

Several caveats that need to be kept in mind when examining Table 6. First, the fish samples are primarily large game fish fillets collected to assess human health concerns. We reported PCB values for fillets of fish >18" as data for this size class existed for all sites which also had sediment PCB data. It should be noted that eagles rarely consume fish this large and generally consume the whole fish, not just the fillet. Therefore the fish PCB data presented does not represent the actual concentrations found in fish that eagles select at a given site. They may provide an index of a site's level of PCB contamination, however. The sediment PCB data should also be viewed cautiously as few sites were sampled extensively. The values presented represent the mean sediment PCB concentration of up to six cores taken at a given site (Fox River, Duluth/Superior Harbor) however most sites had only 2-3 cores taken. Because of varying rates sediment deposition/transport, a few samples may greatly over- or under- represent the contamination level at a given site.

Therefore, to more accurately assess the relationship between nestling plasma PCB concentrations and that in the food it consumes, a determination of the size and type of prey they consume should be made, and representative samples of that size and species should be collected and analyzed. Sediment cores from several sites within a feeding area would also need to be taken if one wished to accurately assess the relationship between nestling plasma PCB concentrations and sediment contamination.

Nestling plasma contaminant levels at Wisconsin RAP sites

Bald eagle nestling plasma samples were obtained near three Wisconsin RAP sites 1990 - 1992. A nestling was sampled in 1991 at DU-25, L. Pokegama, Douglas Co., and found to have plasma total PCB concentration of 106 ug/L; p,p'-DDE was <IDL (2.5 ug/L). This nest is near the St. Louis River/Duluth Harbor RAP site, approximately 4 miles upstream from the mouth of the St. Louis River. A nestling plasma sample and two addled eggs were collected in 1992 from MT-16, Hwy BB, Marinette Co., 4 miles from the Marinette RAP site. The plasma is currently being analyzed at Michigan State University. The addled eggs are frozen and archived with the WDNR. In addition, 3 nestling plasma samples were collected from OU-1a, Kaukauna, on the lower Fox River; 2 in 1991 and 1 in 1992. This nest is 10 miles downstream from Little Lake Butte des Mortes, a primary source of sediment PCBs for the lower Fox River and Green Bay RAP site. Plasma PCBs in 1991 = 124 & 115 ug/L, p,p'-DDE = <IDL in both. The 1992 samples is currently being analyzed. An addled egg was also collected at OU-1a in 1990, the Wisconsin Lab of Hygiene determined that the egg contained 38 ppm total PCB, 1 ppm DDE. An aliquot of the sample has been sent to the USFWS East Lansing office for AHH induction assay. Finally, a plasma sample was collected from a nestling at OC-4, Oconto River, in 1990. The sample is currently being analyzed. While the nest is 25 miles north of the Green Bay RAP site, these nestlings are also likely exposed to elevated level of OCs in the Green Bay estuary. Periodic sampling of nestlings and addled eggs at these nests and any new nests near

RAP sites (BR-1, Little Tail Point, Brown County has a subadult & adult in residence in 1992, this nest is within 10 miles of the Green Bay RAP site) can provide a means for assessing contaminant trends and the result of remediation efforts at those sites.

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TABLES

Table 1

Project: Wisconsin DNR--Bald Eagle Plasma Analysis

Aquatic Toxicology Laboratory
201 Pesticide Research Center
Michigan State University
East Lansing, Michigan 48824

Sample	Band Number	Year	Nest #	Location	Previous No. p,p'-DDE ng/ml	Total PCB ng/ml
1	629-35441	1990	MT 8	GRAND RAPID POWER PLANT	[3.7]	67
2	629-35437	1990	MT 8	GRAND RAPID POWER PLANT	<IDL	54
3	629-35431	1990	MT 11	TWIN ISLAND	10	105
4	629-35432	1990	MT 11	TWIN ISLAND	23	213
5	629-35433	1990	MT 11	TWIN ISLAND	13	136
6	629-35297	1990	OC 2	OCONTO R./BATTLE CREEK	7	38
7	629-35298	1990	OC 3	W. PESHTIGO R.	10	49
8	629-35208	1990	AS 12	N. TWIN	17	41
9	629-35332	1990	AS 16	BASSWOOD ISLAND	43	135
10	629-35333	1990	AS 28	LONG ISLAND	15	56
11	629-35334	1990	BY 23A	ORIENTA FLOWAGE	30	102
12	629-35300	1990	IR 11	RICE LAKE	17	59
13	629-35315	1990	IR 33	TFF DAM	7	27
14	629-35319	1990	IR 25A	EAGLE ISLAND	[2.6]	18
15	629-35321	1990	IR 34	SPRINGSTED LANDING	[4.9]	47
16	629-35323	1990	IR 8C	MCGIVERS PASS	[2.7]	32
17	629-35325	1990	IR 27	CEDAR LAKE	9	66
18	629-35327	1990	IR 19	OXBOW LAKE	12	35
19	629-35328	1990	IR 9C	MURRAYS LANDING	11	52
20	629-35329	1990	IR 3B	BIG PINE LAKE	[3.3]	19
21	629-35331	1990	IR 32	SAXON HARBOR	36	124
22	629-35306	1990	JU 2	YELLOW RIVER	7.3	197
23	629-35308	1990	MA 2A	BIG EAU PLEINE	[4.8]	51
24	629-35311	1990	LI 7B	PINE RIVER	[4.3]	103
25	629-35312	1990	LI 4A	GRANDMOTHER DAM	6.4	147
26	629-31798	1991	MT 8	GRAND RAPIDS POWER PLANT	[3.0]	46
27	629-36322	1991	IR	STURGEON BAY	6	56
28	629-36328	1991	IR 9C	MURRAYS LANDING	6	47
29	629-36330	1991	IR	TWIN LAKES	77	75

Table 1 (Cont.)

30	629-36320	1991	IR 33	TFF DAM	[4.8]	46
31	629-35390	1991	SA 6B	BIG BLOCK	<IDL	40
32	629-35388	1991	RU 5A	LADYSMITH	<IDL	33
33	629-35389	1991	RU11	FLAMBEAU R.	<IDL	27
34	629-35377	1991	OC 1	ARCHIBALD L.	14	37
35	629-35376	1991	OC 3	WAUPEE FLOWAGE	8	19
36	629-35373	1991	SH 1	SHAWANO L.	<IDL	30
37	629-35372	1991	MT 9	HIGH FALLS	<IDL	24
38	629-35369	1991	MT 2	L. NOQUEBAY	8	330
39	629-35392	1991	OU 1	KAUKAUNA/FOX #1	<IDL	124
40	629-35394	1991	OU 1	KAUKAUNA/FOX #2	<IDL	115
41	629-36340	1991	DU 2	AMNICON RIVER	<IDL	79
42	629-36331	1991	AS 16	BASSWOOD ISL.	24	73
43	629-36333	1991	AS 15	OAK ISLAND	20	114
44	629-36334	1991	IR 32	SAXON HARBOR	16	66
45	629-36336	1991	DU 9	BRULE MOUTH	15	70
46	629-35395	1991	DU 25	L. POKEGAMA	<IDL	106
47	629-36338	1991	BY 21	BARK RIVER	<IDL	85
48	Not on Vial	1991	AS 26	DEVILS ISL. #1	11	43
49	Not on Vial	1991	AS 26	DEVILS ISL. #2	10	83
50	Not on Vial	1991	AS 12	N. TWIN ISL.	42	201
51	Not on Vial	1991	AS 3	OUTER ISLAND	56	198
52	629-36318	1991	VI 89	EAGLE RIVER	<IDL	24
53	629-36326	1991	ON 8	SWAMP CREEK	<IDL	59
54	629-36316	1991	ON 25	BACKWATER BAR	[4.4]	31
55	629-35399	1991	LI 7	PINE RIVER	8	118
56	629-35365	1991	MA 3	HALF MOON L.	<IDL	156
57	629-35363	1991	WO 2	SCHMIDT SLOUGH	<IDL	187
58	629-35360	1991	PO 3	STEVENS POINT	6	31
59	629-35359	1991	JU 2	YELLOW RIVER	<IDL	100
60	629-35357	1991	AD 1	CASTLE ROCK	9	277

[n] = Below level of quantification (LOQ) for method
<IDL = Below instrument detection level (IDL) of 2.5 ng/ml
IDL = 2.5 ng/ml, p,p'-DDE; 5 ng/ml, PCBs
LOQ = 5 ng/ml, p,p'-DDE; 10 ng/ml, PCBs for 2 ml plasma extracts

Table 2. Wisconsin 1990-91 nestling bald eagle plasma contaminant concentrations, by location.

		total PCB (ug/L)			p,p'-DDE (ug/L)		
Location	N	% with Detect-able	Geo-Metric Mean	Range	% with Detect-able	Geo-Metric Mean	Range
I.	16	100	84	41-201	81	16	<IDL-56
II.	1	Sample analysis underway at MSU					
III.	13	100	47	18-75	100	6	3-77
IV.	13	100	46	19-330	77	7	<IDL-23
V.	11	100	125	31-277	58	4	<IDL-9
VI.	7	100	33	24-59	17	<IDL	<IDL-5

Location I - <8 km from Lake Superior
Location II- <8 km from Lake Michigan
Location III - counties adjacent to Lake Superior & > 8 km inland
Location IV - counties adjacent to Lake Michigan & > 8 km inland
Location V - inland counties, historic point source discharge
Location VI - inland counties, no historic point source discharge

Table 3. Mean concentrations and ranges (ug/L) of PCBs and DDE from nestling bald eagles in the Great Lakes Basin[1].

		Total PCBs		DDE	
Nesting Area	n	Mean	Range	Mean	Range
Great Lakes	42	183.3	33.0-520.0	60.9	13.0-306.0
Interior	79	23.7	5.0-200.0	20.0	2.0-193.0

[1] Bowerman et al. 1990

Table 4. Mean concentrations and ranges (ug/L) of PCBs and DDE in whole blood of nestling bald eagles from Oregon and Washington[1]. Concentrations have been corrected by a conversion factor of two to be equivalent to blood plasma values.

Breeding Area	n	Total PCBs Mean	Total PCBs Range	DDE Mean	DDE Range
Lower Columbia River	14	129.0	0.0-351.0	98.0	66.2-130.5
Outer Klamath Basin	24	22.0	0.0-580.0	44.0	nc[3]
Upper Klamath Lake	17	0.0	nd[2]-280.0	46.0	nc[3]
Cascade Lakes	34	0.0	nd[2]-280.0	20.0	nc[3]

[1] Frenzel, 1985; Garrett, et al. 1988.

[2] nd = not detected in sample.

[3] nc = not given in citation.

Table 5 1992 Blood Collection Territories in Wisconsin

Territory	Date	Band #	Weight (kg)	Sex	Blood Coll. (cc)
WO-2c	5/29/30	629-36342	NW	F	10
JU-2a	5/29/92	629-36344	3.65	M	10
AD-1	5/29/92	No Band[1]	NW	Unk.	10
OU-1a	5/30/92	629-36346	3.66	F	10
OU-1a	5/30/92	629-36347	3.43	F	10
MT-16	5/30/92	629-36349	2.60	F	9
SA-14a	6/2/92	629-36310	2.94	F	10
WA-34	6/2/92	629-36312	3.05	M	10
SA-60e	6/2/92	629-36313	3.91	F	10
SA-65	6/2/92	629-36314	4.30	F	9
BA-3a	6/3/92	629-36315	4.72	F	10
WA-11	6/3/92	629-37362	3.44	M	10
VI-65b	6/4/92	629-36356	3.57	M	11
BY-23	6/7/92	629-36360	3.40	M	10
BY-21	6/7/92	629-36362[2]	3.35	M	10
AS-25	6/7/92	629-36363	4.30	F	10
BY-15a	6/7/92	629-36365	4.25	F	10
IR-9c	6/9/92	629-36369	3.81	F	10
IR-33	6/9/92	629-36370	4.15	F	10
DU-18	6/10/92	629-36358	4.65	F	10
SA-27a	6/10/92	629-36373	3.80	M	10
DU-25b	6/11/92	629-36375	4.50	F	10
IR-32a	6/12/92	629-36377	3.20	M	10
VI-61c	6/13/92	629-36366	3.15	M	10
VI-57	6/13/92	629-36380	NW	M	10
DU-02g	6/16/92	629-36381	4.35	F	10
DU-24a	6/17/92	629-36382	4.34	F	10
AS-24a	6/18/92	629-36384	3.60	M	10
DU-14a	6/18/92	629-36385	4.20	F	10
IR-35a	6/19/92	629-36362[2]	3.15	M	10
AS-20c	6/22/92	629-36387	3.85	M	10
AS-25	6/24/92	629-36389	4.65	F	10
	6/27/92	629-36386	3.90	M	10

[1]This eaglet was captured on the ground after it had fledged, the bander was not present. Sample and measurements were taken.

[2]This band was first placed on the eaglet at nest BY-21. Several days after banding the eaglet was found dead below the nest tree. The band was recovered and placed on the chick at nest IR-35a (Blair Lake).

Table 6. Wisconsin bald eagle nestling plasma, sediment, and fish fillet total PCB content, by location.

Location	Nestling plasma total PCB (ug/L)	Fish (>18") fillet PCB ug/g fresh Mean (range)	Sediment PCB (0-5 cm) ug/g dry Mean (range)
Duluth/ Superior Harbor	106	0.7 (<0.2-1.1)	0.2 (0.1-0.6)
L. Superior (Douglas)	70, 79	0.3 (<0.2-0.6)	NM
L. Superior (Bayfield)	85, 102	ND	NM
L. Superior (inner APIS[1])	56, 135	0.2 (<0.2-0.5)	NM[2]
L. Superior (outer APIS[3])	41, 43, 83, 198, 201	1.3 (<0.2-5.8)	NM
L. Fox River/ Kaukauna	115, 124	2.5 (0.4-9.8)	2.6 (0.5-4.8)
Menominee R./ Marinette[4]	213, 136, 105	1.6 (0.3-3.0)	0.1 (0.1-0.2)
Wisconsin R. (Castle Rock/ Petenwell)	100, 187, 197, 277	0.6 (0.3-1.3)	ND[5]
Wisconsin R. (Stevens Pt)	31	0.8 (0.3-1.9)	0.2
Wisconsin R. (Mosinee- L. Du Bay)	156	0.3	0.6 (0.4-0.8)
Wisconsin R. (below Merril)	103, 118	1.0	NM
Wisconsin R. (below Tomahawk)	147	ND	ND
Flambeau R.	27, 33, 37	ND	NM

Table 6 (cont.)

ND - none detected
NM - not measured
[1] - Basswood Island, Oak Island
[2] - samples currently under analysis with US Geological Survey
[3] - Devils, N. Twin, & Outer Islands
[4] - Plasma sampled at Twin Islands, 10 miles upstream from fish and sediment samples (Marinette)
[5] - sediment collected at mouth of tributaries, none collected in deposition zones

FIGURES

Figure 1

NESTLING EAGLE PLASMA SAMPLE LOCATIONS 1990 - 1991

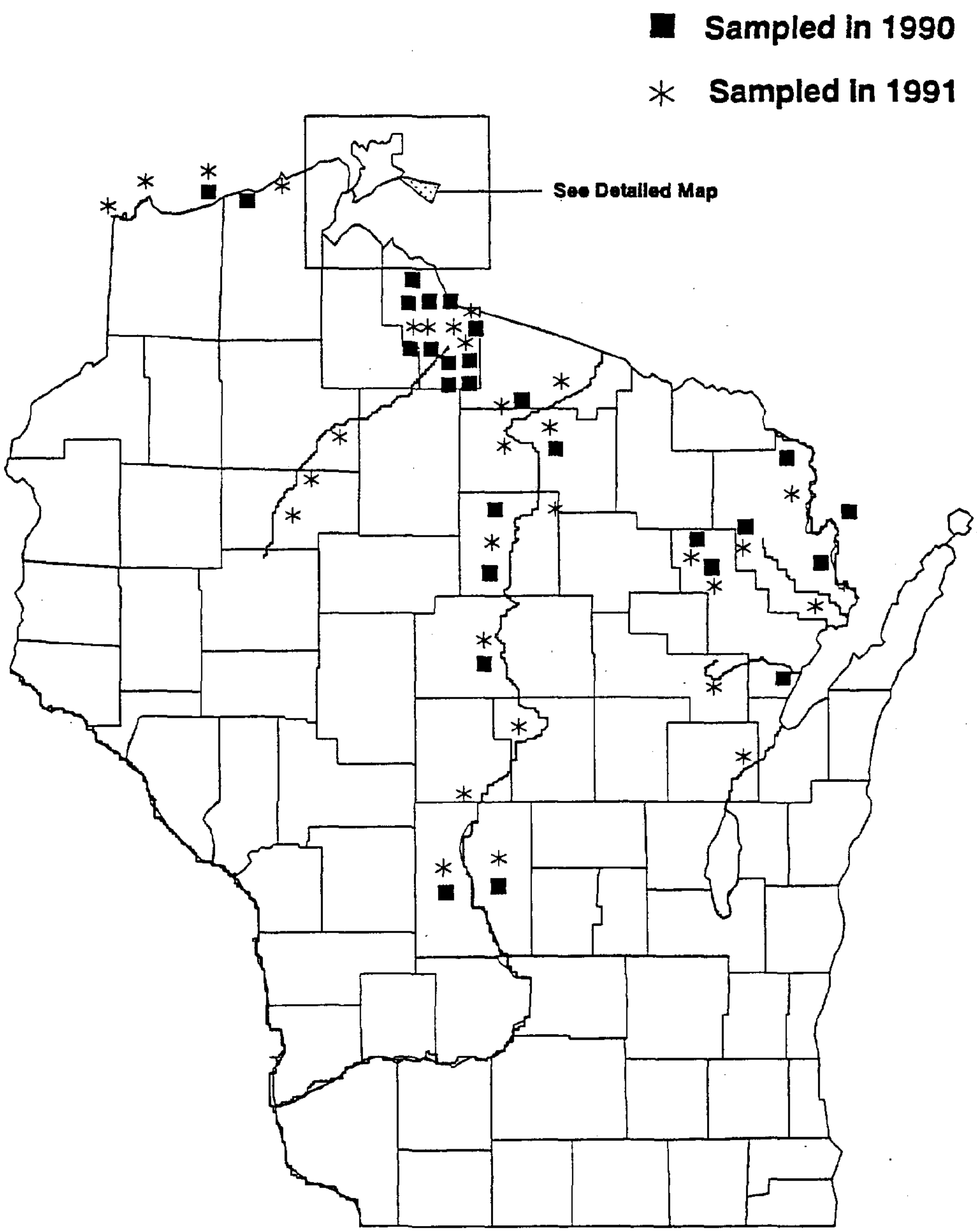


Figure 2

LAKE SUPERIOR REGION
NESTLING EAGLE PLASMA SAMPLES
1990 - 1991

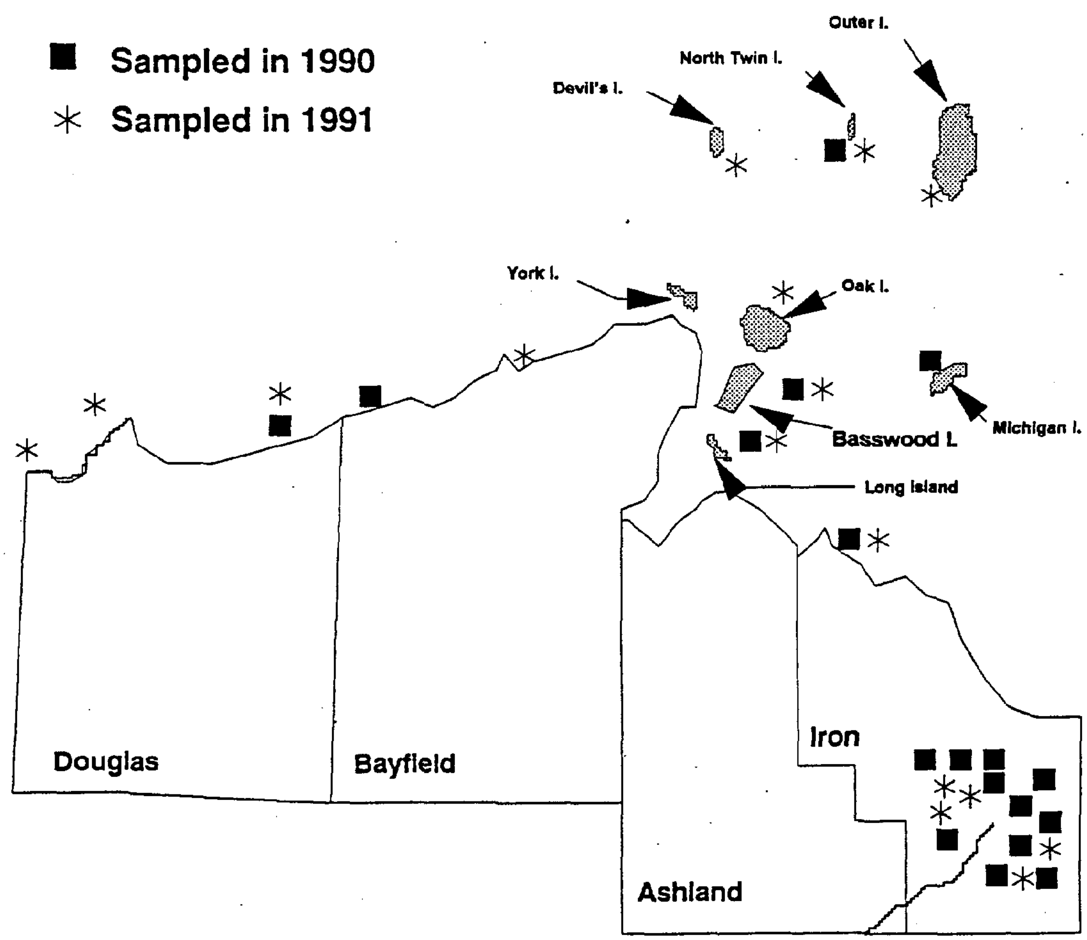


Figure 3

WISCONSIN BALD EAGLE RESEARCH SITES 1992

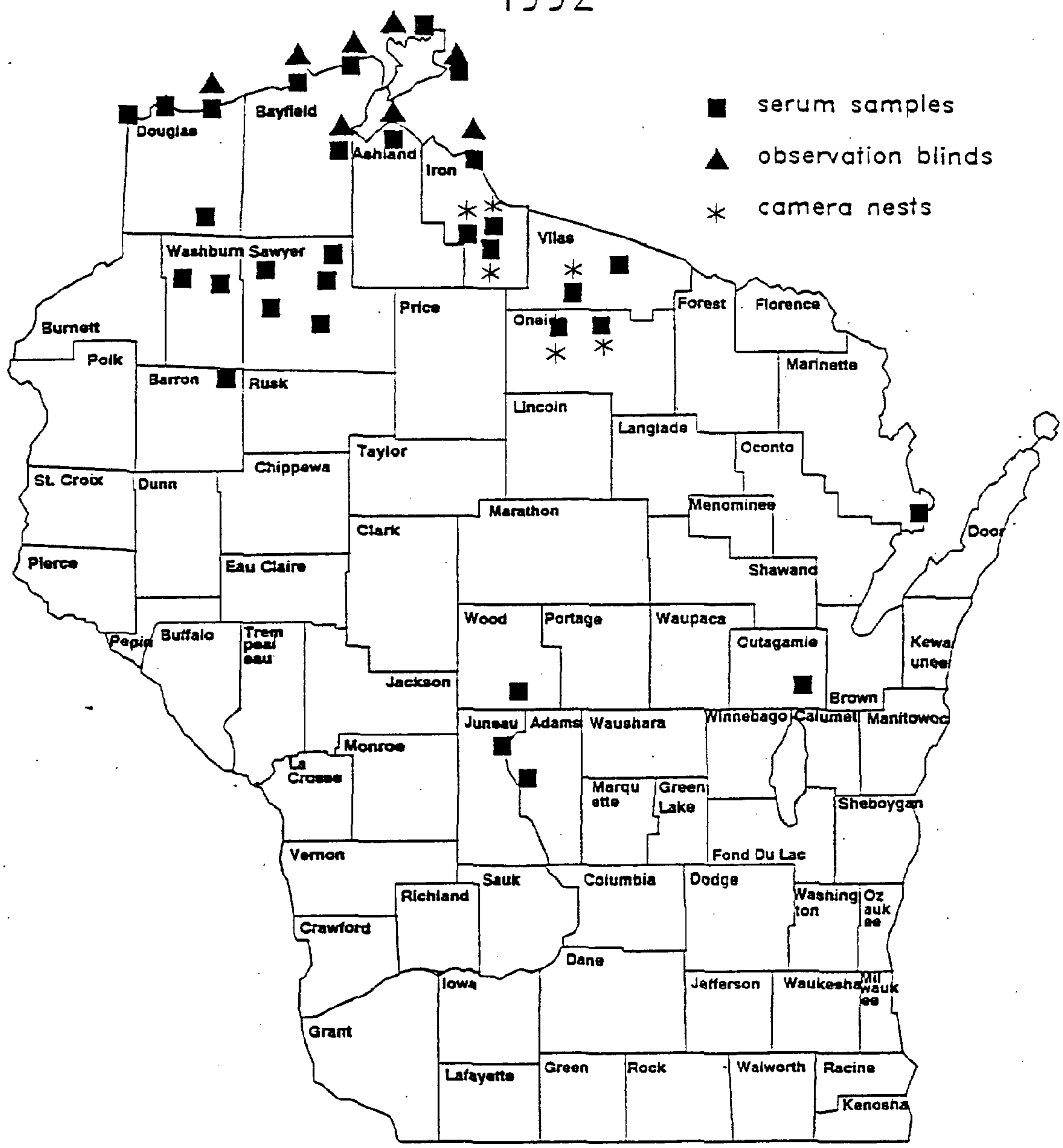


Figure 4

Wisconsin Bald Eagle Nestling Plasma PCB Concentrations
By Location (1990-1991)

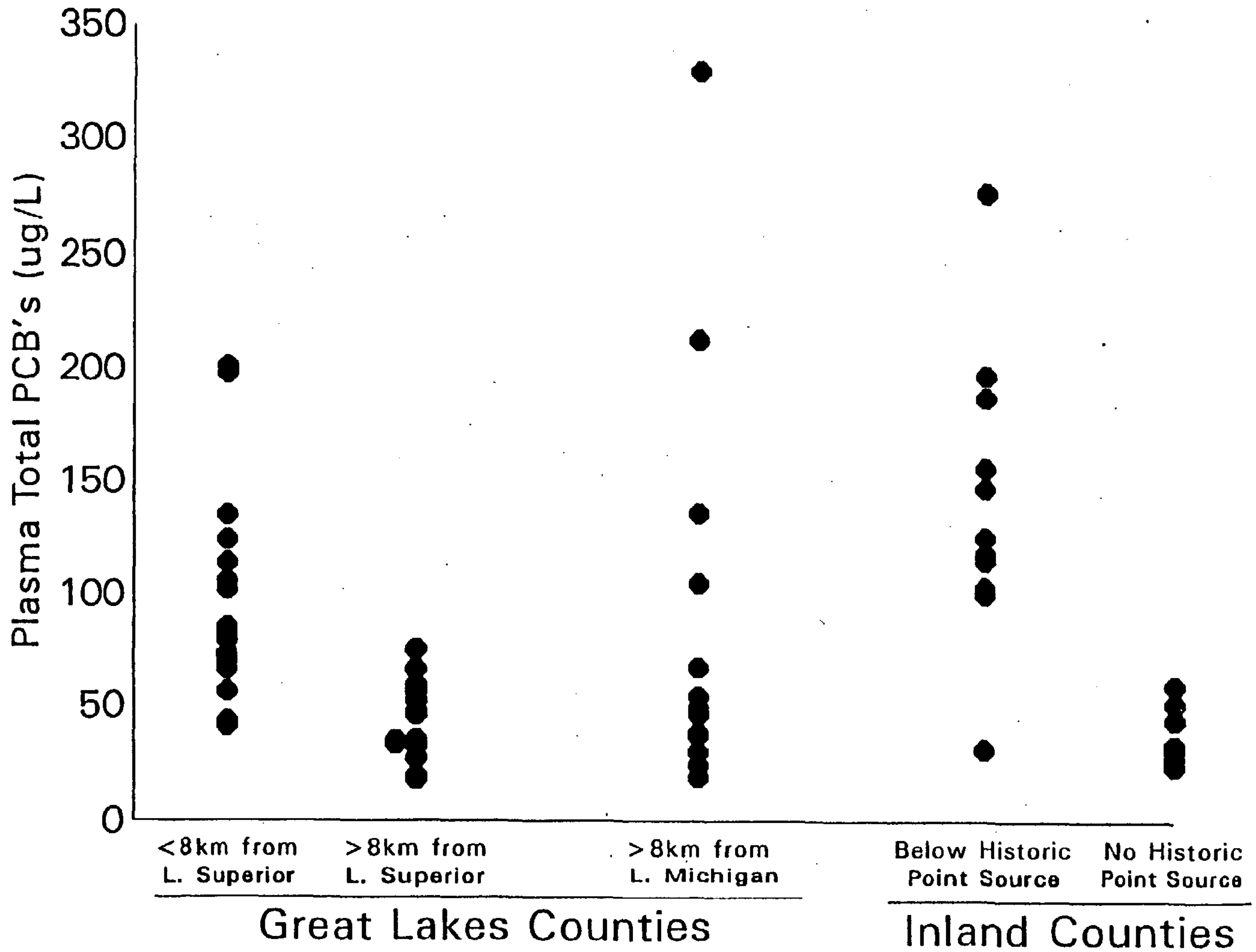


Figure 5

Wisconsin Bald Eagle Nestling Plasma DDE Concentrations By Location (1990-1991)

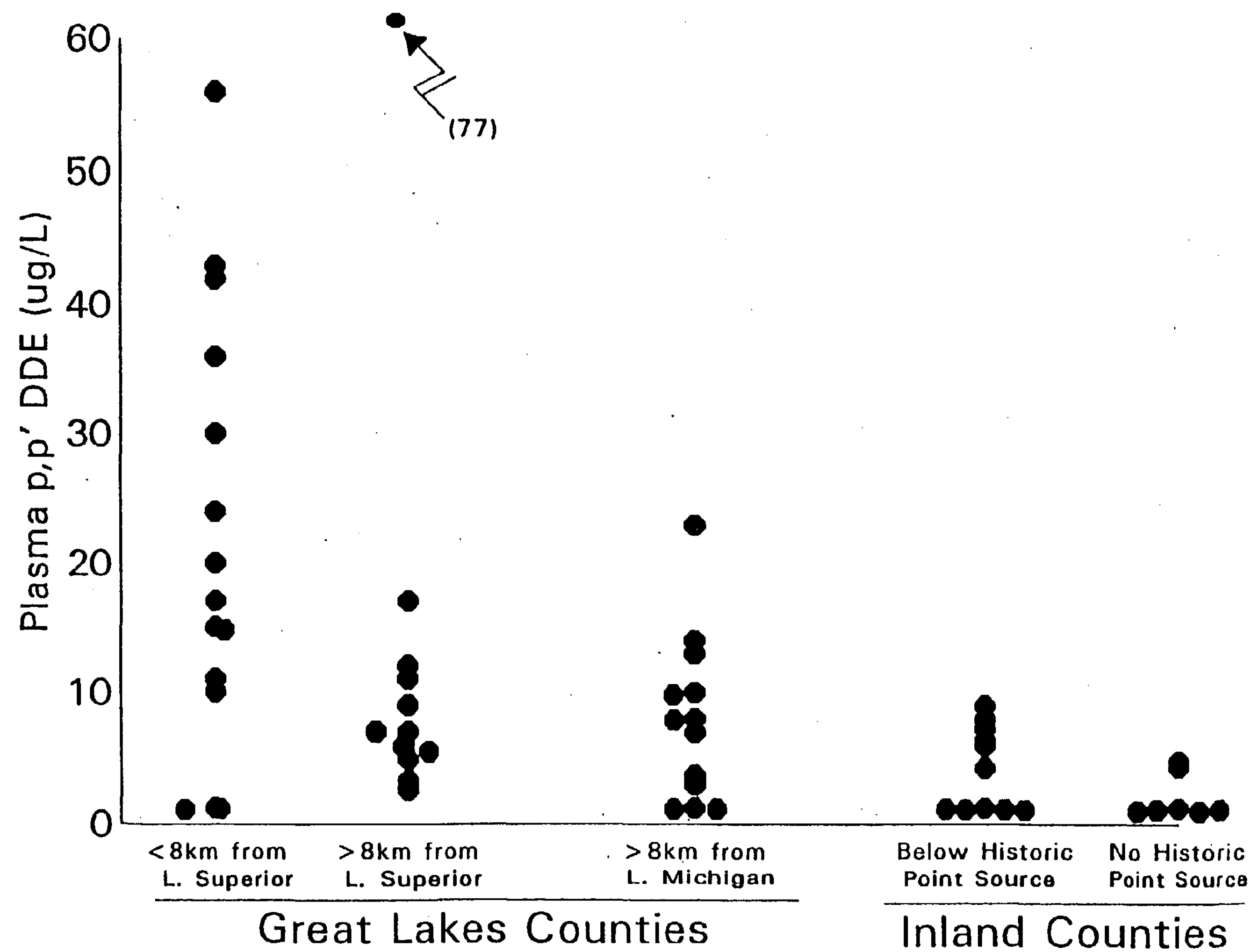
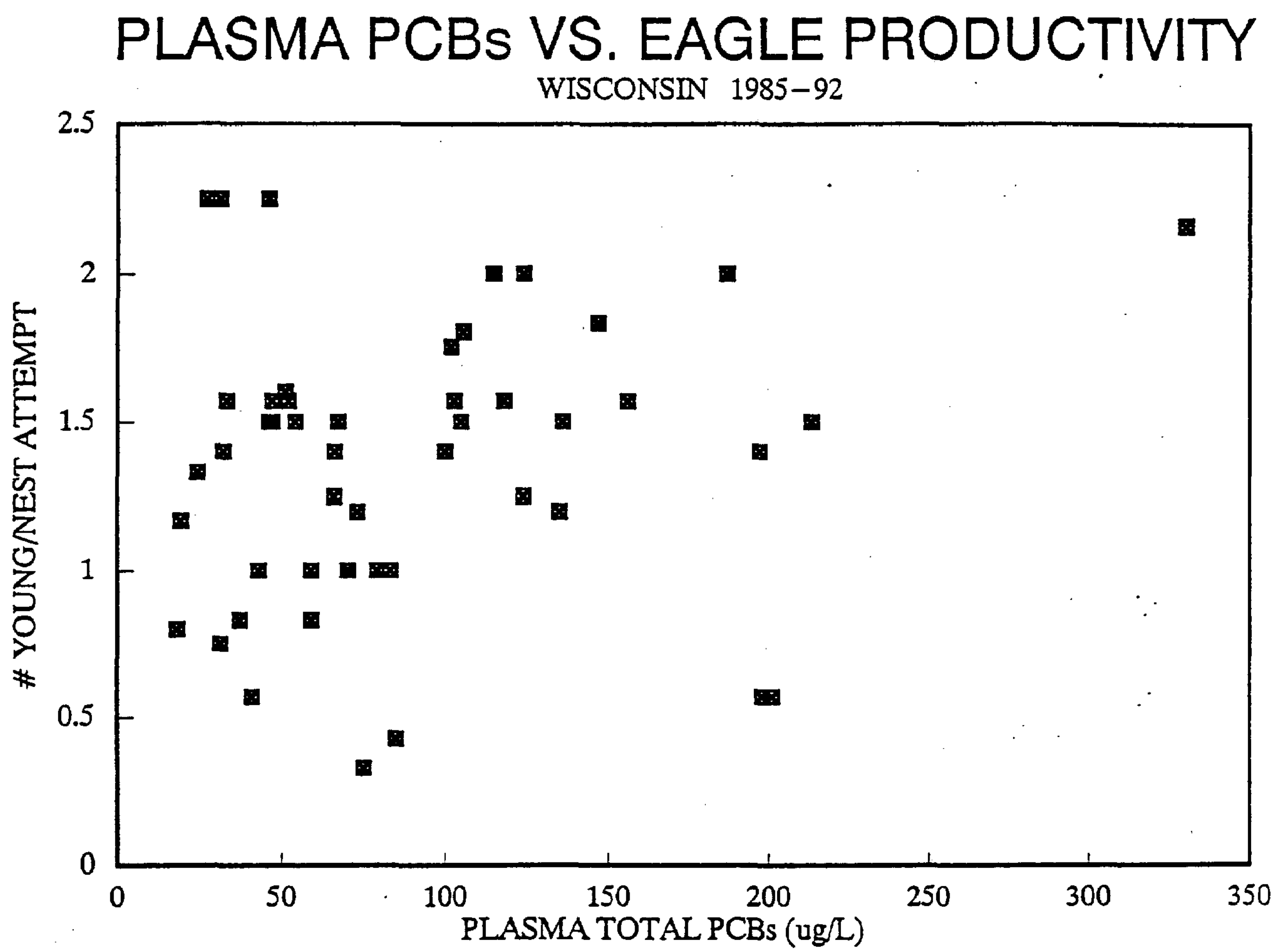


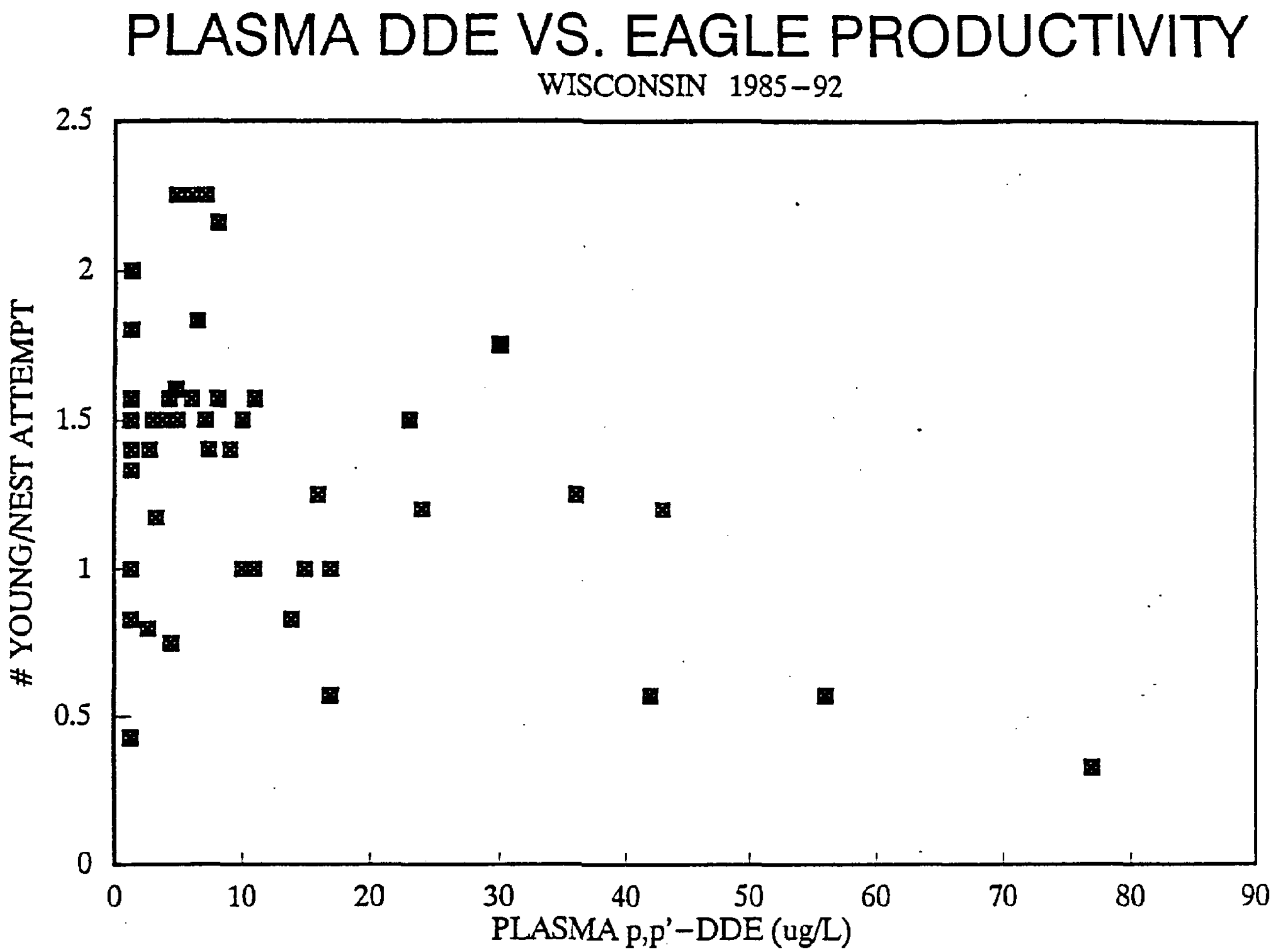
Figure 6



PLASMA PCBs VS. YOUNG/NEST ATTEMPT

Regression Output:		
Constant		1.253711
Std Err of Y Est		0.484551
R Squared		0.025469
No. of Observations		48
Degrees of Freedom		46
X Coefficient(s)	0.001219	
Std Err of Coef.	0.001112	

Figure 7



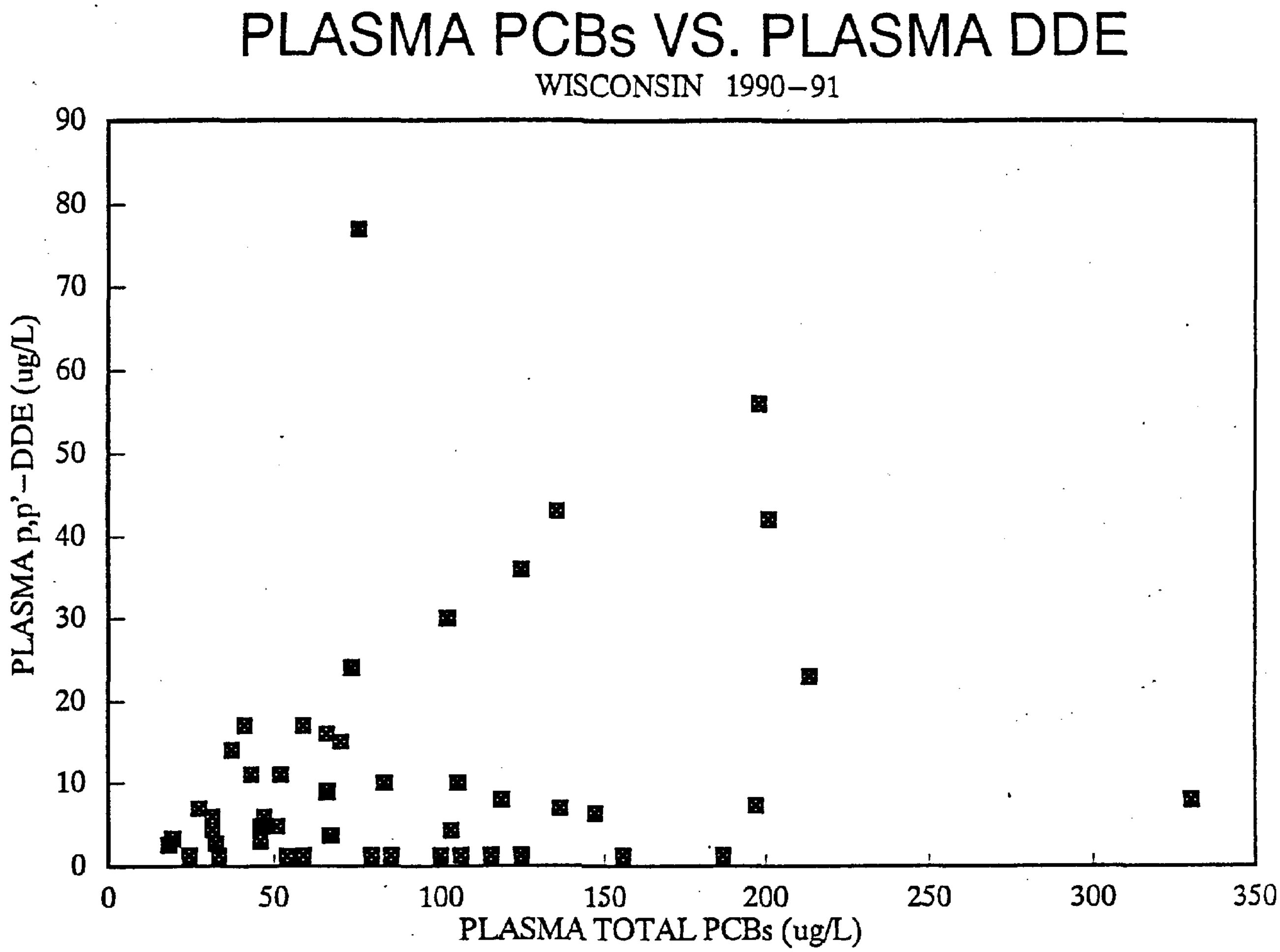
PLASMA DDE VS YOUNG/NEST ATTEMPT

Regression Output:

Constant	1.536152
Std Err of Y Est	0.434254
R Squared	0.217284
No. of Observations	48
Degrees of Freedom	46

X Coefficient(s)	-0.01445
Std Err of Coef.	0.004043

Figure 8



PLASMA PCBs VS. PLASMA DDE

Regression Output:

Constant		71.83647
Std Err of Y Est		63.69064
R Squared		0.071792
No. of Observations		60
X Coefficient(s)	1.226053	
Std Err of Coef.	0.57887	

Figure 9

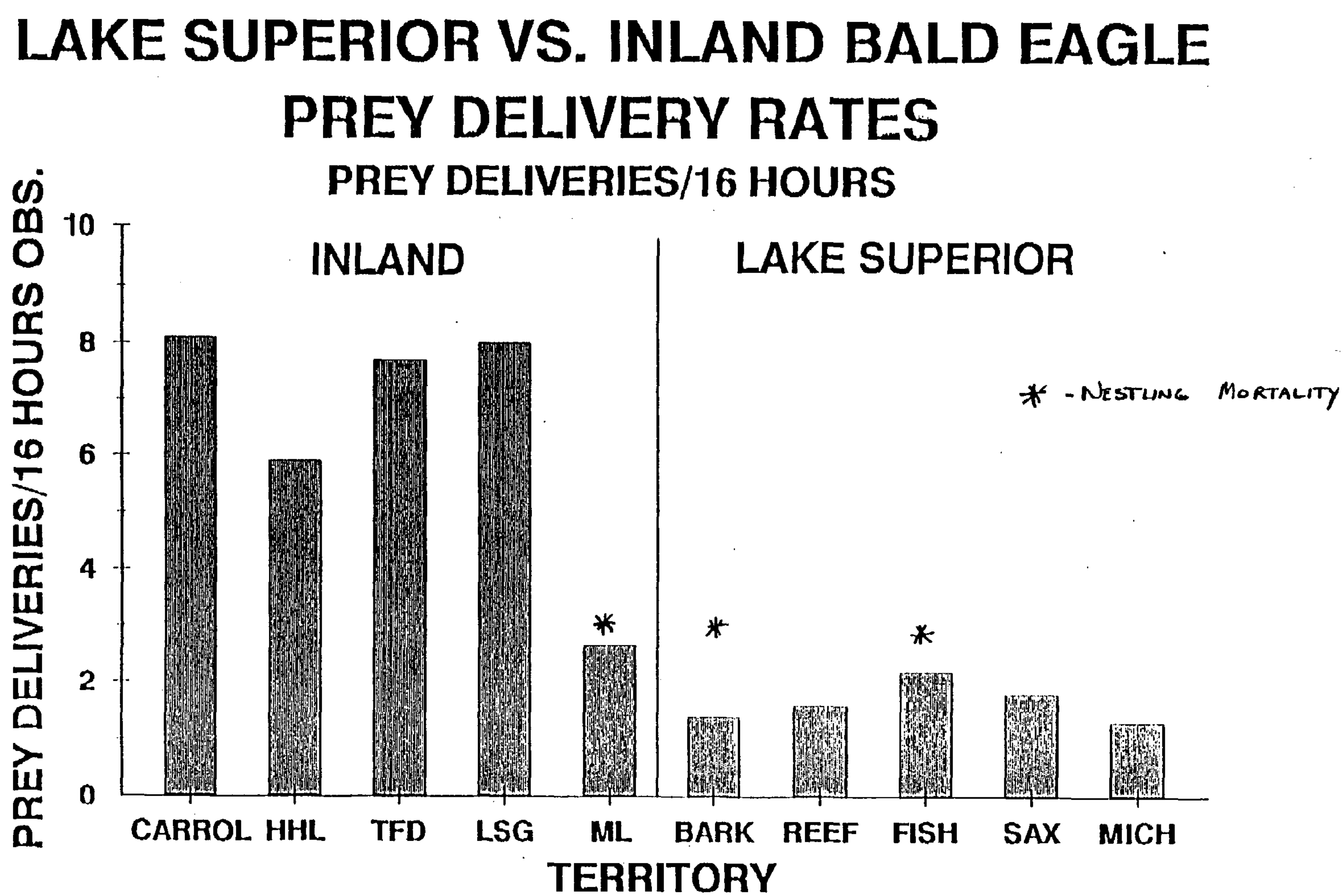

LAKE SUPERIOR VS. INLAND BALD EAGLE
PREY DELIVERY RATES
PREY DELIVERIES/16 HOURS
PREY DELIVERIES/16 HOURS OBS.
10
8
6
4
2
0
INLAND
LAKE SUPERIOR
* - NESTLING MORTALITY
*
*
*
CARROL
HHL
TFD
LSG
ML
BARK
REEF
FISH
SAX
MICH
TERRITORY

Figure 10

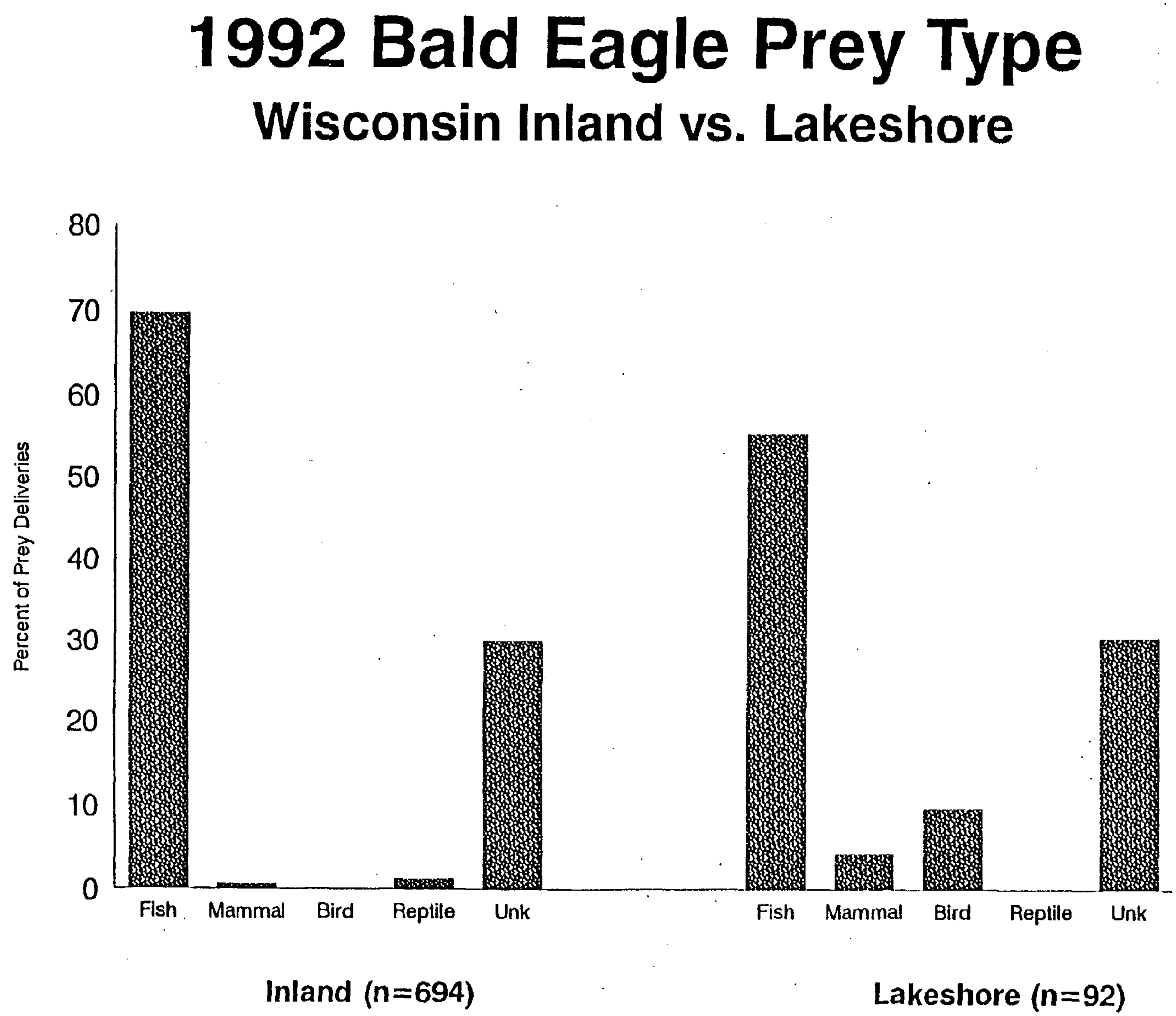

1992 Bald Eagle Prey Type
Wisconsin Inland vs. Lakeshore
Percent of Prey Deliveries
80
70
60
50
40
30
20
10
0
Fish
Mammal
Bird
Reptile
Unk
Fish
Mammal
Bird
Reptile
Unk
Inland (n=694)
Lakeshore (n=92)

Figure 11

1992 Bald Eagle Prey Size Classes

Wisconsin Inland vs. Lakeshore

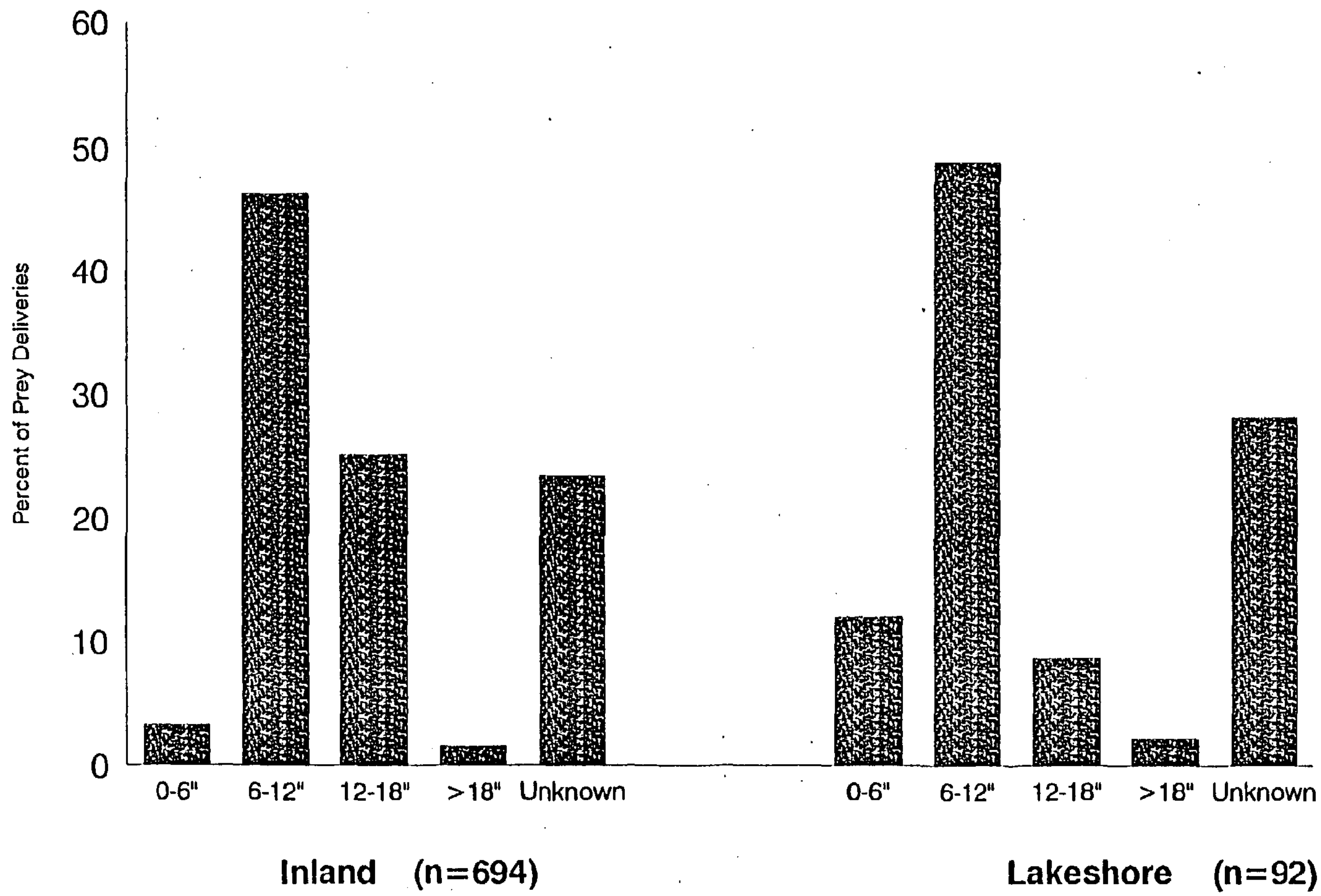
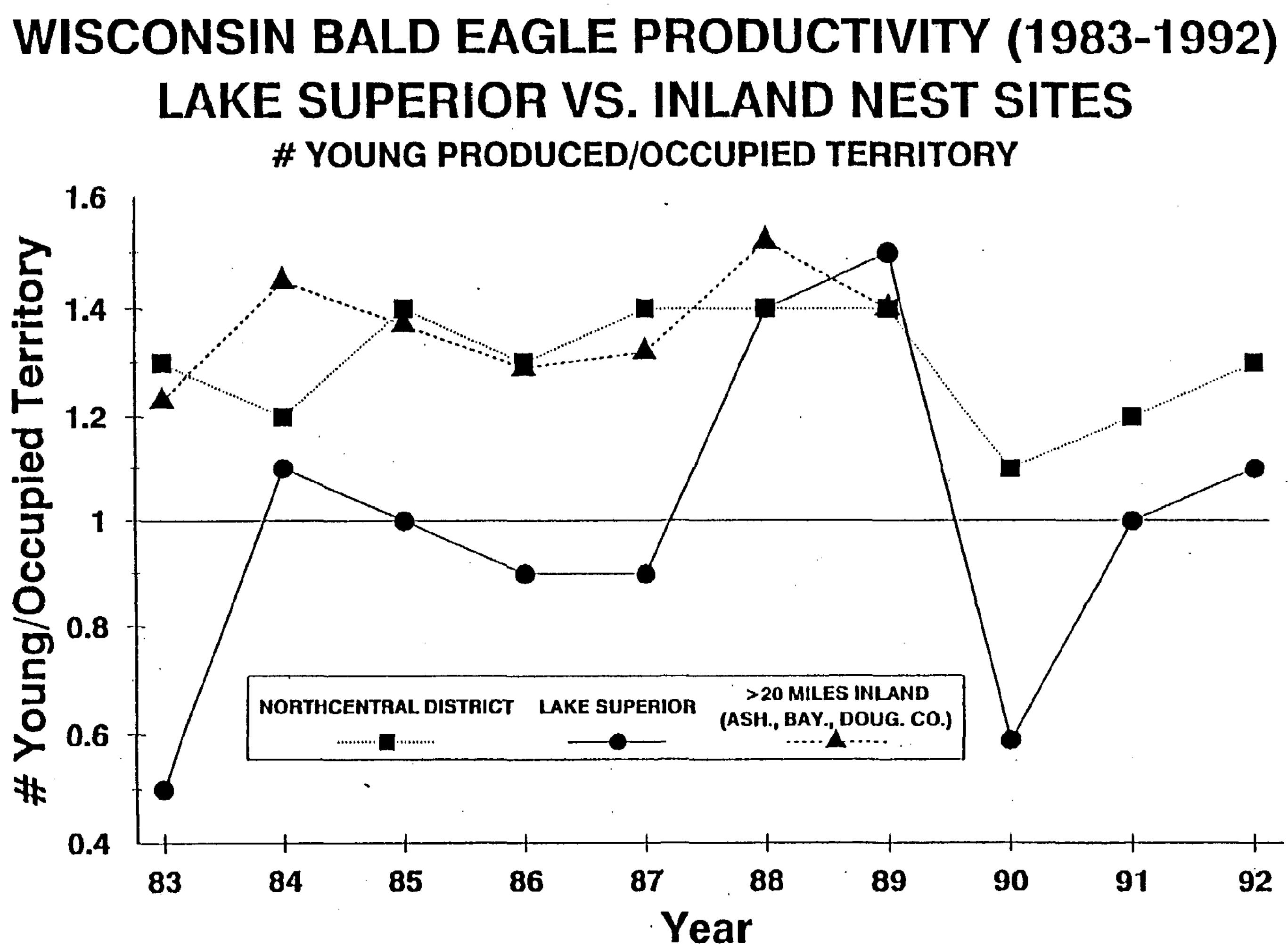


Figure 12


WISCONSIN BALD EAGLE PRODUCTIVITY (1983-1992)
LAKE SUPERIOR VS. INLAND NEST SITES
YOUNG PRODUCED/OCCUPIED TERRITORY
Young/Occupied Territory
1.6
1.4
1.2
1
0.8
0.6
0.4
83
84
85
86
87
88
89
90
91
92
Year
NORTHCENTRAL DISTRICT
LAKE SUPERIOR
>20 MILES INLAND (ASH., BAY., DOUG. CO.)

APPENDIX

MICHIGAN STATE UNIVERSITY

PESTICIDE RESEARCH CENTER
TELEPHONE (517) 353-6376

EAST LANSING • MICHIGAN • 48824-1311
FAX: (517) 353-5598

December 23, 1992

Dr. Micheal Meyer
Wisconsin DNR
Bureau of Research
1350 Femrite Road
Monona, WI 53716

Dear Dr. Meyer,

I am enclosing the Quality Assurance Report for the Wisconsin DNR--Bald Eagle Plasma Analysis. If I can be of further assistance, my number is (517) 336-2028.

Sincerely,

Christine Vandervoort

Christine Vandervoort

MICHIGAN STATE UNIVERSITY

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QUALITY ASSURANCE REPORT

STUDY: Wisconsin DNR--Bald Eagle Plasma Analysis
Cooperative Research with Wisconsin DNR and Michigan State University
Aquatic Toxicology Laboratory

STUDY DIRECTOR: Dr. John P. Giesy

STUDY FACILITY: Michigan State University
Aquatic Toxicology Laboratory
E. Lansing, MI 48824

DATE OF REPORT: December 23, 1992

I have reviewed the PCB and DDE anayltical chemistry work done on the Bald Eagle Plasma collected in Wisconsin. The work was performed in Dr. John Giesy's laboratory at Michigan State University. The following is my opinion of the study mentioned above.

The analysis was performed according to the SOP for the analysis by personnel of appropriate training and experience. The data analysis was done by William Bowerman and confirmed by Dave Verbrugge, Head Chemist for Dr. John Giesy. My review of data analysis agrees with the data presented.

The study appears to have been performed as stated in the work plan. If you have any questions I may be contacted at (517) 336-2028.

Report Written by: Christine Vandervoort, QAO
Pesticide Research Center
Michigan State University
E. Lansing, MI 48824

SOP No._____

Michigan State University
Pesticide Research Center
Aquatic Toxicology Laboratory

STANDARD OPERATING PROCEDURE

ANALYSIS OF ORGANOCHLORINE PESTICIDES and PCBs IN BIRDS' PLASMA

Prepared by: Miguel A. Mora
Dave Verbrugge

I. SCOPE

The scope of this method is to determine the concentrations of organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in plasma of wild birds. A list of the compounds that can be determined by this method and the individual detection limits are given in Table 1. The extraction and cleanup procedures are adapted from Sonzogni et al. (1991), Burse et al. (1990), Schmitt et al. (1985), and SOP from Michigan Department of Public Health (1987) with some modifications. The method's precision is within 10% and the accuracy >90%. Total PCBs are reported as a mixture of Aroclors 1242, 1248, 1254, and 1260. Chromatograms of standard mixtures are given in Appendix. The instrument detection limit (IDL), method detection limit (MDL), and method quantitation limit have been determined as described in Taylor (1989).

II. REFERENCES

Burse, V.W., S.L. Head, M.P. Korver, P.C. McClure, J.F. Donahue, and L.L. Needham. 1990. Determination of selected organochlorine pesticides and polychlorinated biphenyls in human serum. J. Anal. Toxicol. 14:137-142.

Michigan Department of Public Health. 1987. Analysis of blood for polychlorinated and polybrominated biphenyls, and chlorinated hydrocarbon pesticides. Analytical Method No 7. Lansing, Michigan.

Monro, A.M. 1990. Interspecies comparisons in toxicology: The utility and futility of plasma concentrations of the test substance. Reg. Toxicol. Pharmacol. 12:137-160.

Schmitt, C.J., J.L. Zajicek, and M.A. Ribick. 1985. National pesticide monitoring program: Residues of organochlorine chemicals in freshwater fish, 1980-81. Arch. Environ. Contam. Toxicol. 14:225-260.

Sonzogni, W., L. Maack, T. Gibson, D. Degenhardt, H. Anderson, and B.

Fiore. 1991. Polychlorinated biphenyl congeners in blood of Wisconsin sport fish consumers. Arch. Environ. Contam. Toxicol. 20:56-60.

Taylor, J.K. 1989. Quality Assurance of Chemical Measurements. Lewis Publishers, Inc., Chelsea, Michigan, 328 pp.

III. SUMMARY

This method permits the separation of organochlorine pesticides and PCBs from small volumes of birds' plasma. One ml plasma fractions are denaturized with methanol, extracted with a 1:1 mixture (v/v) of hexane-ethyl ether and cleaned up with mixed solvents in florisil and silica gel columns. The florisil and silica gel fractions are analyzed by gas chromatography with electron capture detector (GC-ECD). OCs and PCBs are confirmed by GC/MS in 10% of the samples. Average recoveries for a mixture of 6 pesticides were 77.5% (88% without dieldrin) and % for PCBs.

IV. SIGNIFICANCE AND USE

The determination of organochlorine pesticides and PCBs in plasma of wild birds is of importance for the understanding of the relationships of the distribution of xenobiotics between plasma and body tissues. The plasma reaches many target organs and xenobiotics are removed from the plasma as this goes through a given tissue (Monro 1990). By being able to determine concentrations of organochlorine pesticides and PCBs in plasma we can accomplish the following: first, we can take samples of individual species without having to kill them; and second, the same individuals can be sampled over time allowing us to determine seasonal variations and bioaccumulation of contaminants in marked individuals.

V. INTERFERENCES

There are components in plasma that may produce some interference. This can be avoided by following an adequate cleanup procedure. Interferences will be detected by comparing the environmental samples with periodical runs of chicken plasma blanks. Prior to initiating the studies the lab facility will be carefully cleaned to reduce contamination risks. Chicken plasma (obtained from the chicken farm MSU) will be used as control and for recovery experiments.

VI. APPARATUS

Gas chromatograph, Perkin Elmer, model 8500, with electron capture detector (ECD) with ^{63}Ni foil at 350°C. Column: DB-1 fused silica capillary column (J & W Scientific), 30 m x 0.25 mm i.d., 0.25µm film thickness. Injector in splitless mode. Septum purge set at 3-5 ml per minute, temperature at 220°C. Carrier gas: helium at 5 psig, flow rate of @ 1 ml per minute. Makeup gas nitrogen at 45 psig. Total flow rate of @ 50 ml/min. Autosampler Perkin Elmer 8300. Data (retention times and area percentages) are transferred directly to a microcomputer.

VII. REAGENTS AND MATERIALS

A. Reagents: Methanol, hexane, diethyl ether (peroxide free), petroleum ether, isooctane, benzene, and acetone; Burdick and Jackson, Baxter, Muskegon, Michigan. All solvents used are of high purity or pesticide grade quality.

B. Sodium sulfate, anhydrous, granular and powder forms. Rinse with hexane or methylene chloride in a buchner funnel before use. Let air dry for a while, then dry in the oven at 130^{0}C for at least 24 hr before use. May keep stored at 130^{0}C.

C. Glass wool. Rinse glass wool with methylene chloride or hexane for at least 24 hr before use.

D. Florisil, 60/80 mesh, PR grade, Floridin Co., Pittsburgh, PA. Activate at 130^{0}C for at least 48 hr before use. Keep stored in the oven at 130^{0}C.

E. Silica gel 60, 70/230 mesh. Activate at 130^{0}C for at least 24 hr before use. Store at 130^{0}C.

F. Glassware. All glassware is washed with liquinox detergent, rinsed with tap and deionized water, then rinsed with acetone and hexane before use.

G. Reference standards.

1. Pesticide matrix spike, (3/90) catalog # 32018, Lot # A000071, Restek Corporation, Bellefonte, PA.
2. Certified reference material, PCBs (aroclor 1260) in human serum, lot # SRM1589; National Bureau of Standards (NBS), Gaithersburg, MD.
3. Internal standard, PCB 30, obtained from Acustandards. Stock and working solutions were prepared in our lab.
4. Aroclors 1242, 1248, 1254, and 1260, obtained from Dr. Zabik, Pesticide Research Center, MSU, originally obtained from Monsanto Co. Stock and working solutions were prepared in our lab.
5. Chlorinated hydrocarbon pesticides: Analytical reference standards obtained from U.S. EPA, Quality Assurance Division, Research Triangle Park, NC. Stock and working solutions and mixtures were prepared in our lab.
6. Bovine serum and plasma reference material, obtained from Department of Public Health, Lansing, Michigan (MDPH).
7. EPA human plasma for blind analysis (interlab. studies), obtained from MDPH.

VIII. HAZARDS AND PRECAUTIONS

Some of the solvents used are flammable and explosive. Solvents should be always used under the hood and away from fire. Use of lab coats and eye protection goggles is important. In case of a spill, skin contact or inhalation problems, follow specifications in material safety data sheets (MSDS). Handling and storage precautions should follow the recommendations described in the respective MSDS. Waste should be collected and disposed properly according to MSU ORCBS indications.

IX. SAMPLING AND SAMPLE PREPARATION

Blood from the brachial vein of fish-eating birds (caspian terns, double-crested cormorants and bald eagles) was collected in heparinized tubes according to specified procedures. Each sample was marked with bird's common name, location, date and band number (if banded). The samples were either centrifuged in the field (10 min at 3000 rpm), or placed in a refrigerator (4^0 C) and centrifuged within 48 hours. The plasma was separated from the red blood cells and stored in the freezer until chemical analysis.

X. PREPARATION OF APPARATUS

Prior to use the instrument performance is mostly determined from previous runs. Check pressures of He at 5 psig, N_2 at 45 psig. If gases are not on, turn make-up gas on, then turn auxiliary pressure control knob to suggested psig. Percent saturation is adjusted to 0.9%. If the baseline is appropriate, then we can assume that the working conditions are optimal. Column performance should be determined from previous recent runs, and by injecting standards before the autosampler run. The autosampler is loaded and the QC run is set in the computer. Set computer to receive information from each run, then set output (GC) to external device.

XI. CALIBRATION AND STANDARDIZATION

Availability and use of appropriate standards: Our pesticide laboratory standards have been evaluated with the use of a certified pesticide matrix spike, catalog # 32018, lot # A000071, Restek Corporation, Bellefonte, PA. The relative response factors obtained for the two sets of standards were within ___.

The performance of the GC will be monitored daily by measuring the response and retention times of several calibration mixes. The number of theoretical plates will be calculated using two compounds, C20-ATA and 2,4,6-trichlorobiphenyl (IUPAC #30). The ratio of the theoretical plates (#30/C20) will be used to monitor the condition of the column. A record of the retention times, peak responses, theoretical plates, and peak shape will be kept in the GC Log book. If the theoretical plate ratio changes by > Ÿ25% from its mean value, or if serious column deterioration is observed, the column may be replaced if the situation cannot be corrected. If the retention time of any internal standard changes by > 0.5 min from its mean value, the system will be checked and corrected as required.

The linear range of the GC will be established for pesticide mixtures and for a 1:1:1:1 mix of Aroclors 1242, 1248, 1254 and 1260 using a performance relative response factor (PRRF). The PRRF is defined by the equation (ex. for aroclor);

$$PRRF = AR_{total area} * ISTD_{conc} / AR_{conc} * ISTD_{area}$$

AR= Aroclor Mix ISTD= Internal Standard
total area= sum of peak areas for the aroclor mix
area= peak area for ISTD
conc= concentration in ng/µl

The PRRF is specific for each OC or for a 1:1:1:1 mixture of Aroclors, and is used only to monitor instrument performance. The PRRF will be constant over the linear range of the detector. Constant is defined as Ý3% from the mean value for the PRRF. This range will encompass a minimum of 1.5 orders of magnitude using a minimum of 3 concentrations. The target operating linear range will be 5, 2.5, and 0.15ng of Aroclor mix injected, and 0.25, 0.1, and 0.01 ng for OCs. Once the linear range has been established, an individual standard solution for each of the mixtures will be chromatographed. These chromatographs will be used as templates for pesticide mixtures and the Comstar PCB pattern recognition program. The integrity of the template will be checked by daily injection of pesticide mixtures and a 1:1:1:1 Aroclor performance standard. The absolute concentration of the performance standard will be adjusted to the linear range of the instrument. The calculated concentration of the mix should be Ý10% of the expected value.

Calibration checks will be run at the beginning and end of a sample set, where a set is approximately 10 samples. If the concentration of the standard mix is outside of the 10% range the template will be rechromatographed prior to further sample analysis.
A log of the relative response factors (RRF) for the individual Aroclors will also be maintained as a check of the GC performance over the course of the study. The RRF is defined by the equation:

$$RRF = AR_{total area} * ISTD_{conc} / AR_{conc} * ISTD_{area}$$

AR= Individual Aroclor ISTD= Internal Standard
total area= sum of peak areas for the aroclor
area= peak area for ISTD
conc= concentration in ng/µl

If the RRF for a given pesticide or aroclor changes by > 10% from its mean value, the instrument will be checked and the appropriate maintenance (ie. bakeout, clean detector, etc.) will be completed before prior continuing with the analyses. The standards should be rechromatographed and new templates prepared.

XII. PROCEDURE

A. SAMPLE extraction.

1) Transfer 1 ml of plasma to a 10 ml test tube with teflon cap. Record also plasma weight by difference from test tube weight.

2) Add 0.5 ml methanol and vortex for about five seconds.

3) Extract with 5 ml of hexane-ethyl ether (1:1, v/v) by agitation in a burrel-wrist action shaker for 10 min.

4) Transfer extract to centrifuge tube and centrifuge at 2000 rpm for 5 min. Transfer extract to a second centrifuge tube to combine the extract volumes and further evaporation.

5. Repeat extraction procedure (steps 3 and 4) twice (three times total).

6. Add 0.5 ml of isooctane, then concentrate extract to 0.5 ml in a rotary evaporator or N-evap on a warm water bath.

B. FLORISIL cleanup and fractionation.

1) Prepare columns by placing 1 cm of granular anhydrous Na_2SO_4 on glasswool in a 1 cm x 30 cm i.d. chromatography column fitted with a 250 ml reservoir. Add five grams of 60/80 mesh Florisil and top with another 1 cm layer of sodium sulfate.

2) Wash each column with 20 ml of petroleum ether and discard resulting effluent.

3) When petroleum ether reaches the top of the Na_2SO_4 layer, add the concentrated extract (approx. 0.5 ml) and allow it to drain into the column. Rinse the flask at least three times with @ 1 ml of petroleum ether each time. Transfer the rinses into the column and discard the eluent resulting from loading and rinsing.

4) Wash the column walls with 5 ml of 6:94 ratio of diethyl ether:petroleum ether and collect the eluent in a 250 ml round-bottom flask. When the solvent reaches the top of the Florisil add another 30 ml of the 6:94 solvent and continue collection. Set this fraction aside for silica gel fractionation.

5) Repeat the above procedure using a 20:80 ratio of diethyl ether:petroleum ether in place of the 6:94 solution and collect the eluent from the 5 ml wash + 35 ml elution in a second 250 ml flask.

6) Rotary evaporate the two resulting fractions to about 1 ml.

7) Transfer the 20% fraction (containing dieldrin, endrin,

methoxychlor and *o,p*-DDD) to a centrifuge tube with three hexane rinses. Add 0.5 ml of isooctane and then N-evap to 0.5 ml. Bring it up to 1 ml with isooctane and transfer to a 2 ml vial with teflon cap. Rinse the vial previously with acetone, hexane and isooctane. Spike the sample with 50 μl of PCB #30 (11.4 ng/ml) before injection into the GC. Take 300 μl into an autosampler vial and load into autosampler for GC run.

C. SILICA GEL cleanup and fractionation

1) Prepare silica gel 60 (70/230 mesh) columns in the same manner as the florisil column.

2) Wash the column with 20 ml hexane.

3) When hexane reaches the top of the silica gel, add the 6% florisil eluate (1-2 ml) and allow it to drain into the column. Rinse flask with 3 ml of hexane and drain into column. Discard eluents.

4) Wash the column with 5 ml of a 0.5:99.5 ratio of benzene:hexane, followed by 35 ml of the solvent. Collect the eluates in a 250 ml round-bottom flask. (This is fraction 1, silica gel).

5) Elute the columns with 40 ml of a 25:75 ratio of diethyl ether:hexane and collect the eluate in a 250 ml round-bottom flask. (This is fraction 2, silica gel).

6) Rotary evaporate both fractions to about 1 ml, then transfer to a centrifuge tube with three rinses of hexane. Add 0.5 ml of isooctane and N-evap down to @ 0.5 ml. Bring it up to 1 ml with isooctane again and transfer to 2 ml vial with teflon cap. Before GC analysis, spike the extract with 50 μl of PCB #30 (11.4 ng/ml), then take 250 μl into an autosampler vial for GC run.

D. GAS CHROMATOGRAPHY determination.

1. Silica Gel 25% fraction. Most pesticides come out in this fraction. use autosampler/GC program 9.

   Program 9 conditions: Injector temperature 230 ^{0}C, Detector temperature 350 ^{0}C. Gas carrier He at 5 psig, makeup gas nitrogen at 45 psig. Equilibrium time 3 min, Total run time 60 min, attenuation 8.

   Oven temperature program

	1	2	3	4
Oven temp (^{o}C)	120	150	225	280
Iso time (min)	3	5	10	15
Ramp rate (^{o}C/min)	30	4	20	

2. Silica gel 0.5% fraction. PCBs and DDE come out in this fraction. Use autosampler/GC program 6.

Program 6 conditions: Injector and detector temperatures as well as gas flow rates and everything else remains the same as in program 9, except for the oven temperature program and running time.

	Oven temperature program		
	1	2	3
Oven temp (^{o}C)	120	260	280
Iso time (mim)	6	0	0
Ramp rate (^{o}C/min)	2	20	

3. Florisil 20% fraction. Some pesticides come out in this fraction. Use program 9 (see above).

XIII. DEMONSTRATION OF STATISTICAL CONTROL

Statistical control of GC measurements can be demonstrated graphically by the use of control charts (Taylor 1989, p. 129). Initially, a standard of known concentration will be injected for a total of 7 independent measurements. If the range is linear, the mean relative response factor will be used as the central line to maintain statistical control. Standards will be injected every day that a set of samples is run. If the value of the standard is within 1 standard deviation of the mean, then we can say that we have statistical control. If a known reference standard is used, then the certified concentration value can be used as the central line (Taylor 1989, p. 131). The control limits will be evaluated by the control charts.

In addition, for every set of 10 samples one sample will be run in triplicate. The calculated concentrations will be compared. If the CV (coefficient of variation is Ÿ 10%, then we can assume that our measurements are within our established method precision. The use of standards of known concentrations will allow to construct standard reference calibration curves against which the sample runs will be compared. If an outlier is suspected, the calculations and data transfers will be rechecked. If the results are still suspect then the sample before and after suspect and the suspect sample will be reanalyzed. A value will be considered an outlier if there is an assignable cause.

XIV. CALCULATIONS

The concentration of PCBs and OCs will be determined using the internal standard method to eliminate injection variability and the need to maintain the sample at a constant final volume.

A) Organochlorine pesticides: Pesticides will be quantified based on an internal standard (PCB 30) added to the samples after the extraction step. Quantification is carried out by calculating relative response factors based on peak areas.

B) Total PCBs: PCBs will be quantified with the use of COMSTAR (see COMSTAR SOP).

XV. CONFIRMATION AND ASSIGNMENT OF UNCERTAINTY

Organochlorine pesticides will be confirmed in approximately 10% of the samples by GC/MS. This confirmation may only be possible for compounds detected at significant concentrations.

Assignment of uncertainty: A range performance chart will be constructed where the relative response factors (RRFs) at low, middle, and high concentrations will be plotted vs concentration. The upper warning limit (UWL) and lower control limit (LCL) will be the 95% CI, and the upper control limit (UCL) the 99.7% CI. Samples with values above the UCL will be diluted and reanalyzed; those with values below the LCL will be tagged as below detection limit.

TABLE 1

Retention times and limits of detection of OC pesticides[1]

Compound	Retention time (min)	Method detection limit (ng/ml)	Limit of detection (ng/ml)
HCB	14.27		
gamma-HCH	14.93	2.3	0.8
Int.std PCB 30	15.41		
Heptachlor	19.37	1.1	0.4
Aldrin	21.22	1.9	0.6
Heptachlor epoxide	23.06		
Oxychlordane	23.27		
gamma-chlordane	24.17		
o,p'-DDE	24.61		
Endosulfan I	24.79		
p,p'-DDD	25.04		
α-Chlordane	25.49		
Dieldrin	26.06	1.3	0.4
p,p'-DDE	26.12		
t-Nonachlor	26.38		
Endrin	26.93	5.6	1.9
Endosulfan II	27.06		
o,p'-DDD	27.90		
p,p'-DDT	30.06	12.6	4.2
Methoxychlor	33.71		

[1] Column DB-1, Autosampler method 9; see SOP for GC conditions and procedures.